Antibodies Against Protective Antigen

Related Applications

[0001] This application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Nos. 60/391,162, filed June 26, 2002, 60/406,339, filed August 28, 2002, 60/417,305, filed October 10, 2002, 60/426,360, filed November 15, 2002, 60/434,807, filed December 20, 2002, 60/438,004, filed January 6, 2003, 60/443,858 filed January 31, 2003, 60/443,781, filed January 31, 2003, 60/454,613 filed March 17, 2003, and 60/468,651 filed May 8, 2003. Each of the aforementioned applications is hereby incorporated by reference in its entirety.

Field of the Invention

[0002] The present invention relates to antibodies and related molecules that specifically bind to the protective antigen (PA) of *Bacillus anthracis*. Such antibodies have uses, for example, in the prevention, detection and treatment of anthrax and/or anthrax related toxins. The invention also relates to nucleic acid molecules encoding anti-PA antibodies, vectors and host cells containing these nucleic acids, and methods for producing the same. The present invention relates to methods and compositions for preventing, detecting, diagnosing, treating or ameliorating anthrax and/or anthrax related toxins, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that specifically bind to PA.

Background of the Invention

[0003] *Bacillus anthracis* is a Gram-positive, aerobic, spore forming bacterium that is responsible for the deadly disease anthrax. There are three recognized routes of anthrax infection including cutaneous (through skin), gastrointestinal, and pulmonary (via inhalation) infection. Of the three ways to contract the disease, inhalation is the avenue that most frequently leads to the death of the patient.

[0004] Anthrax secretes a deadly three-component exotoxin which is comprised of three proteins, lethal factor (LF), edema factor (EF), and protective antigen (PA). The anthrax toxin is a bipartite toxin that contains A and B moieties, similar to that of

diphtheria toxin and many clostridial toxins. The LF and EF proteins function as enzymatic A moieties of the toxin, while the PA protein functions as the B, or binding, moiety.

[0005] During the process of intoxication, PA binds to its cell surface receptor, (e.g., anthrax receptor (ATR) and/or capillary morphogenesis gene 2 (CMG2)) and is cleaved at the sequence RKKR by cell surface proteases such as furin. This cleavage releases a 20 kilodalton fragment of the PA protein, leaving a 63 kilodalton fragment of the PA protein bound to the cell surface (PA63). Some cleavage to the PA63 form may be mediated by serum proteases and occur prior to PA, in this case PA63, binding to the cell surface. Release of the 20 kilodalton PA fragment enables the PA63 fragment to multimerize into a heptameric ring structure and exposes a site on PA63 to which LF and EF bind with high affinity. The complex is then internalized by receptor-mediated endocytosis. Acidification of the vesicle causes conformational changes in the pA63 heptamer that result in transportation of LF and EF toxins across the endosomal membrane, after which they are released into the cytosol where they exert their cytotoxic effects. The edema factor (EF) component of edema toxin (EF+PA) is a calmodulin dependent adenylate cyclase whose action upsets cellular water homeostasis mechanisms, thereby resulting in swelling of infected tissues. The lethal factor (LF) moiety of lethal toxin (LF+PA) is a zinc metalloproteinase that inactivates mitogen activated protein kinase kinase in vitro. Lethal factor induces a hyperinflammatory condition in macrophages resulting in the production of proinflammatory cytokines including TNF-alpha and interleukin-1beta, which are responsible for shock and death of anthrax patients. For more detailed reviews of *Bacillus Anthracis* infection and anthrax toxin please see, e.g., *Critical Reviews in Microbiology* (2001) 27:167-200, *Medical Progress (1999) 341:815-826, and Microbes and Infection* (1999) 2:131-139, each of which are hereby incorporated by reference in their entireties.

[0006] There is a clear need, therefore, for identification and characterization of compositions, such as antibodies, that influence the biological activity of anthrax toxins.

Summary of the Invention

[0007] The present invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that

specifically bind to a PA polypeptide (SEQ ID NO:2) or polypeptide fragment or variant of PA.

[0008] The present invention relates to methods and compositions for preventing, treating or ameliorating anthrax disease and/or symptoms induced by anthrax related toxins (such as lethal toxin or edema toxin) comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that specifically bind to PA or a fragment or variant thereof. In specific embodiments, the present invention relates to methods and compositions for preventing, treating or ameliorating a disease or disorder associated with PA function, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that specifically bind PA or a fragment or variant thereof.

[0009] In other embodiments, antibodies of the invention have a bactericidal effect on *B. anthracis* bacteria. By way of non-limiting example, antibodies of the invention may activate the classical complement pathway and/or enhance the activation of the alternative complement pathway which can lead to killing of bacterial cells. Alternatively, antibodies of the invention may opsonize *B. anthracis* bacteria. Opsonized bacteria then may be a target for antibody dependent cell-mediated cytotoxicty (ADCC). In another embodiment, antibodies of the invention may catalyze the generation of hydrogen peroxide from singlet molecular oxygen and water which chemical reaction results in the efficient killing of bacteria.

[0010] In specific embodiments, antibodies of the invention are administered in combination with other therapeutics or prophylactics such as a soluble form of an anthrax receptor (e.g., SEQ ID NO:3, described in *Nature* (2002) 414:225-229 (which is hereby incorporated by reference in its entirety), e.g., a polypeptide comprising amino acids 1-227 or 41-227 of SEQ ID NO:3) or a soluble form of the CMG2 receptor (SEQ ID NO:42, described in Scobie et al., *Proceedings of the National Academy of Sciences USA* (2003) 100:5170-5174 which is hereby incorporated by reference in its entirety, e.g., a polypeptide comprising amino acids 33-318 of SEQ ID NO:42). Other therapeutics or prophylactics that may be administered in combination with an antibody of the present invention include mutant forms of PA such as the EF/LF translocation deficient forms of PA described in International Publication Number WO01/82788 and in Science (2001) 292:695-697, both of which are hereby incorporated by reference in their entireties. Other

therapeutics or prophylactics that may be administered in combination with an antibody of the present invention include peptide inhibitors that block LF binding to PA such as the P1 peptide, or its polyvalent form described in Nature Biotechnology (2002) 19:958-961 which is hereby incorporated by reference in its entirety. Still other therapeutics or prophylactics that may be administered in combination with an antibody of the present invention include, but are not limited to antibiotics, anthrax vaccines, antibodies immunoreactive with LF, EF or other protein moieties of *Bacillus anthracis*.

[0011] Another embodiment of the present invention includes the use of the antibodies of the invention as a diagnostic tool to monitor the presence of PA.

[0012] Single chain Fv's (scFvs) that specifically bind PA polypeptide (SEQ ID NOS:48-65) have been identified. Thus, the invention encompasses these scFvs, listed in Table 1. In addition, the invention encompasses cell lines engineered to express antibodies corresponding to these scFvs which are deposited with the American Type Culture Collection ("ATCC") as of the dates listed in Table 1 and given the ATCC Deposit Numbers identified in Table 1. The ATCC is located at 10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for Purposes of Patent Procedure.

[0013] Further, the present invention encompasses the polynucleotides encoding the scFvs, as well as the amino acid sequences encoding the scFvs. Molecules comprising, or alternatively consisting of, fragments or variants of these scFvs (e.g., VH domains, VH CDRs, VL domains, or VL CDRs having an amino acid sequence of the corresponding region of the recombinant antibody expressed by a cell line contained in an ATCC Deposit referred to in Table 1), that specifically bind to PA or fragments or variants thereof are also encompassed by the invention, as are nucleic acid molecules that encode these antibodies and/or molecules. In specific embodiments, the present invention encompasses antibodies, or fragments or variants thereof, that bind to an epitope that comprises the RKKR sequence of amino acid residues 193 to 196 of SEQ ID NO:2). In other embodiments, the antibodies of the invention bind an epitope of PA and occlude access of proteases to the RKKR cleavage site of PA (amino acid residues 193 to 196 of SEQ ID NO:2). In other embodiments, antibodies of the invention neutralize the ability of PA to bind to a cellular anthrax receptor, e.g., ATR (SEQ ID NO:3) or CMG2 (SEQ ID NO:42). In other embodiments, antibodies of the invention neutralize the ability of the PA

(particularly the PA63 form of PA) to form oligomers, and more specifically to form heptamers. And in still other embodiments, antibodies of the invention neutralize the ability of PA (particularly the PA63 form of PA) to bind to either EF or LF (SEQ ID NOs:4 or 5, respectively).

[0014] The present invention also provides anti-PA antibodies that are coupled to a detectable label, such as an enzyme, a fluorescent label, a luminescent label, or a bioluminescent label. The present invention also provides anti-PA antibodies that are coupled to a therapeutic or cytotoxic agent. The present invention also provides anti-PA antibodies that which are coupled, directly or indirectly, to a radioactive material.

[0015] In further embodiments, the antibodies of the invention have a dissociation constant (K_D) of 10^{-7} M or less. In preferred embodiments, the antibodies of the invention have a dissociation constant (K_D) of 10^{-9} M or less.

[0016] In further embodiments, antibodies of the invention have an off rate (k_{off}) of 10^{-3}/sec or less. In preferred embodiments, antibodies of the invention have an off rate (k_{off}) of 10^{-4}/sec or less. In other preferred embodiments, antibodies of the invention have an off rate (k_{off}) of 10^{-5}/sec or less.

[0017] The present invention also provides panels of antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants) wherein the panel members correspond to one, two, three, four, five, ten, fifteen, twenty, or more different antibodies of the invention (e.g., whole antibodies, Fabs, $F(ab')_2$ fragments, Fd fragments, disulfide-linked Fvs (sdFvs), anti-idiotypic (anti-Id) antibodies, and scFvs). The present invention further provides mixtures of antibodies, wherein the mixture corresponds to one, two, three, four, five, ten, fifteen, twenty, or more different antibodies of the invention (e.g., whole antibodies, Fabs, $F(ab')_2$ fragments, Fd fragments, disulfide-linked Fvs (sdFvs), anti-idiotypic (anti-Id) antibodies, and scFvs)). The present invention also provides for compositions comprising, or alternatively consisting of, one, two, three, four, five, ten, fifteen, twenty, or more antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof). A composition of the invention may comprise, or alternatively consist of, one, two, three, four, five, ten, fifteen, twenty, or more amino acid sequences of one or more antibodies or fragments or variants thereof. Alternatively, a composition of the invention may comprise, or alternatively consist of, nucleic acid molecules encoding one or more antibodies of the invention.

[0018] The present invention also provides for fusion proteins comprising an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) of the invention, and a heterologous polypeptide (*i.e.*, a polypeptide unrelated to an antibody or antibody domain). Nucleic acid molecules encoding these fusion proteins are also encompassed by the invention. A composition of the present invention may comprise, or alternatively consist of, one, two, three, four, five, ten, fifteen, twenty or more fusion proteins of the invention. Alternatively, a composition of the invention may comprise, or alternatively consist of, nucleic acid molecules encoding one, two, three, four, five, ten, fifteen, twenty or more fusion proteins of the invention.

[0019] The present invention also provides for a nucleic acid molecule(s), generally isolated, encoding an antibody (including molecules, such as scFvs, VH domains, or VL domains, that comprise, or alternatively consist of, an antibody fragment or variant thereof) of the invention. The present invention also provides a host cell transformed with a nucleic acid molecule of the invention and progeny thereof. The present invention also provides a method for the production of an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof) of the invention. The present invention further provides a method of expressing an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof) of the invention from a nucleic acid molecule. These and other aspects of the invention are described in further detail below.

Brief Description of the Figures

[0020] **Figure 1** illustrates the ability of antibodies PWD0283 and PWD0587 to inhibit the binding of biotinylated PA to ATR.

[0021] **Figure 2** graphically depicts the binding of biotinylated-PA to CHO-K1 cells, J744.A murine macrophages, and human macrophages as determined by flow cytometry. The solid line depicts biotinylated -PA binding to cells; the dashed line depicts the background level.

[0022] **Figure 3** illustrates the ability of two antibodies PWD0283 and PWD0587 to inhibit pore formation by PA protein using the assay described in Example 5.

[0023] **Figure 4** illustrates the ability of antibodies PWD0283 and PWD0587 to inhibit lethal toxin (LT)-mediated cell killing. Data are presented as mean ± SD absorbance at 490 nm.

[0024] **Figure 5** illustrates the effect of prophylactic intravenous administration of PWD0283 and PWD0587 60 minutes prior to exposure of male Fisher 344 rats to Lethal Toxin. CAT 002 is an isotype-matched (IgG1) negative control antibody. A single intravenous injection of PWD0283 or PWD0587 60 minutes prior to injection of lethal toxin provided 100% survival at 24 hours with no apparent ill effects. In contrast, a single injection of the negative control mAb, CAT002, provided no protection with 0% survival and an average TTM of 100 minutes. Vehicle or no study agent also provided no protection with 0% survival and an average TTM of 99 minutes and 91 minutes, respectively.

[0025] **Figure 6** shows the 14 day survival curves of the New Zealand White Rabbits (n=12) that received:

a) no treatment (vehicle) two days prior to;

b) prophylactic treatment (1, 5, 10, or 20 mg/kg sc) two days prior to; or

c) therapeutic treatment (40 mg/kg iv) within 1 hour after

challenge via aerosol inhalation of approximately 195X LD_{50}, of *B. anthracis* spores. Experimental details are described more fully in Example 11. Statistical p-values were obtained from a 2-sided log-rank test. The p-values for the comparison among all groups are < 0.0001, regardless of inclusion or exclusion of the 40 mg/kg iv group in the analysis. The p-values marked in the graph are for the comparison versus the vehicle control group.

[0026] **Figure 7** shows the 28 day survival curves of cynomolgus monkeys (n=10 per group) that received no treatment (vehicle) or prophylactic treatment via subcutaneous administration of anti-PA monoclonal antibody PWD0587 (10, 20 or 40 mg/kg), two days prior to challenge via aerosol inhalation of approximately 186X LD_{50}, of *B. anthracis* spores. Experimental details are described more fully in Example 12. Statistical p-values were obtained from a 2-sided log-rank test. The P values for the comparison among all groups are < 0.0001. The P values marked in the graph are for the comparison versus the vehicle control group.

Detailed Description of the Invention

Definitions

[0027] The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds an antigen. As such, the term

antibody encompasses not only whole antibody molecules, but also antibody multimers and antibody fragments, as well as variants (including derivatives) of antibodies, antibody multimers and antibody fragments. Examples of molecules which are described by the term "antibody" herein include, but are not limited to: single chain Fvs (scFvs), Fab fragments, Fab' fragments, $F(ab')_2$, disulfide linked Fvs (sdFvs), Fvs, and fragments comprising or alternatively consisting of, either a VL or a VH domain. The term "single chain Fv" or "scFv" as used herein refers to a polypeptide comprising a VL domain of antibody linked to a VH domain of an antibody.

[0028] Antibodies of the invention include, but are not limited to, monoclonal, multispecific, human or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention), intracellularly-made antibodies (i.e., intrabodies), and epitope-binding fragments of any of the above. The immunoglobulin molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass of immunoglobulin molecule. Preferably, an antibody of the invention comprises, or alternatively consists of, a VH domain, VH CDR, VL domain, or VL CDR having an amino acid sequence of any one of the cell lines in the ATCC Deposits referred to referred to in Table 1, or a fragment or variant thereof. In a preferred embodiment, the immunoglobulin is an IgG1 isotype. In another preferred embodiment, the immunoglobulin is an IgG4 isotype. Immunoglobulins may have both a heavy and light chain. An array of IgG, IgE, IgM, IgD, IgA, and IgY heavy chains may be paired with a light chain of the kappa or lambda forms.Antibodies of the invention may also include multimeric forms of antibodies. For example, antibodies of the invention may take the form of antibody dimers, trimers, or higher-order multimers of monomeric immunoglobulin molecules. Dimers of whole immunoglobulin molecules or of F(ab')2 fragments are tetravalent, whereas dimers of Fab fragments or scFv molecules are bivalent. Individual monomers withon an antibody multimer may be identical or different, i.e., they may be heteromeric or homomeric antibody multimers. For example, individual antibodies within a multimer may have the same or different binding specificities.

[0029] Multimerization of antibodies may be accomplished through natural aggregation of antibodies or through chemical or recombinant linking techniques known in the art. For example, some percentage of purified antibody preparations (e.g., purified IgG1 molecules) spontaneously form protein aggregates containing antibody homodimers,

and other higher-order antibody multimers. Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art. For example, heterobifunctional crosslinking agents including, but not limited to, SMCC [succinimidyl 4-(maleimidomethyl)cyclohexane-1-carboxylate] and SATA [N-succinimidyl S-acethylthio-acetate] (available, for example, from Pierce Biotechnology, Inc. (Rockford, IL)) can be used to form antibody multimers. An exemplary protocol for the formation of antibody homodimers is given in Ghetie et al., Proceedings of the National Academy of Sciences USA (1997) 94:7509-7514, which is hereby incorporated by reference in its entirety. Antibody homodimers can be converted to Fab'2 homodimers through digestion with pepsin. Another way to form antibody homodimers is through the use of the autophilic T15 peptide described in Zhao and Kohler, The Journal of Immunology (2002) 25:396-404, which is hereby incorporated by reference in its entirety.

[0030] Alternatively, antibodies can be made to multimerize through recombinant DNA techniques. IgM and IgA naturally form antibody multimers through the interaction with the mature J chain polypeptide (e.g., SEQ ID NO:44). Non-IgA or non-IgM molecules, such as IgG molecules, can be engineered to contain the J chain interaction domain of IgA or IgM, thereby conferring the ability to form higher order multimers on the non-IgA or non-IgM molecules. (see, for example, Chintalacharuvu et al., (2001) Clinical Immunology 101:21-31. and Frigerio et al., (2000) Plant Physiology 123:1483-94., both of which are hereby incorporated by reference in their entireties.) IgA dimers are naturally secreted into the lumen of mucosa-lined organs. This secretion is mediated through interaction of the J chain with the polymeric IgA receptor (pIgR) on epithelial cells. If secretion of an IgA form of an antibody (or of an antibody engineered to to contain a J chain interaction domain) is not desired, it can be greatly reduced by expressing the antibody molecule in association with a mutant J chain that does not interact well with pIgR (e.g., SEQ ID NOS:45-47; Johansen et al., *The Journal of Immunology* (2001) 167:5185-5192 which is hereby incorporated by reference in its entirety). Expression of an antibody with one of these mutant J chains will reduce its ability to bind to the polymeric IgA receptor on epithelial cells, thereby reducing transport of the antibody across the epithelial cell and its resultant secretion into the lumen of mucosa lined organs. ScFv dimers can also be formed through recombinant techniques known in the art; an example of the construction of scFv dimers is given in Goel et al., (2000) Cancer Research 60:6964-6971 which is hereby incorporated by reference in its

entirety. Antibody multimers may be purified using any suitable method known in the art, including, but not limited to, size exclusion chromatography.

[0031] By "isolated antibody" is intended an antibody removed from its native environment. Thus, an antibody produced by, purified from and/or contained within a hybridoma and/or a recombinant host cell is considered isolated for purposes of the present invention.

[0032] Unless otherwise defined in the specification, specific binding by an antibody to PA means that an antibody binds PA but does not significantly bind to (i.e., cross react with) proteins other than PA, such as other proteins in the same family of proteins). An antibody that binds PA protein and does not cross-react with other proteins is not necessarily an antibody that does not bind said other proteins in all conditions; rather, the PA-specific antibody of the invention preferentially binds PA compared to its ability to bind said other proteins such that it will be suitable for use in at least one type of assay or treatment, i.e., give low background levels or result in no unreasonable adverse effects in treatment. It is well known that the portion of a protein bound by an antibody is known as the epitope. An epitope may either be linear (i.e., comprised of sequential amino acids residues in a protein sequences) or conformational (i.e., comprised of one or more amino acid residues that are not contiguous in the primary structure of the protein but that are brought together by the secondary, tertiary or quaternary structure of a protein). Given that PA-specific antibodies bind to epitopes of PA, an antibody that specifically binds PA may or may not bind fragments of PA and/or variants of PA (e.g., proteins that are at least 90% identical to PA) depending on the presence or absence of the epitope bound by a given PA-specific antibody in the PA fragment or variant. Likewise, PA-specific antibodies of the invention may bind species orthologues of PA (including fragments thereof) depending on the presence or absence of the epitope recognized by the antibody in the orthologue. Additionally, PA-specific antibodies of the invention may bind modified forms of PA, for example, PA fusion proteins. In such a case when antibodies of the invention bind PA fusion proteins, the antibody must make binding contact with the PA moiety of the fusion protein in order for the binding to be specific. Antibodies that specifically bind to PA can be identified, for example, by immunoassays or other techniques known to those of skill in the art, *e.g.*, the immunoassays described in the Examples below.

[0033] Antibodies of the invention may also include multimeric forms of antibodies. For example, antibodies of the invention may take the form of antibody dimers, trimers, or higher-order multimers of monomeric immunoglobulin molecules. Dimers of whole immunoglobulin molecules or of $F(ab')_2$ fragments are tetravalent, whereas dimers of Fab fragments or scFv molecules are bivalent. Individual monomers within an antibody multimer may be identical or different, i.e., they may be heteromeric or homomeric antibody multimers. For example, individual antibodies within a multimer may have the same or different binding specificities. Multimerization of antibodies may be accomplished through natural aggregation of antibodies or through chemical or recombinant linking techniques known in the art. For example, some percentage of purified antibody preparations (e.g., purified IgG1 molecules) spontaneously form protein aggregates containing antibody homodimers, and other higher-order antibody multimers. Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art. For example, heterobifunctional crosslinking agents including, but not limited to, SMCC [succinimidyl 4-(maleimidomethyl)cyclohexane-1-carboxylate] and SATA [*N*-succinimidyl *S*-acethylthio-acetate] (available, for example, from Pierce Biotechnology, Inc. (Rockford, IL)) can be used to form antibody multimers. An exemplary protocol for the formation of antibody homodimers is given in Ghetie et al., *Proceedings of the National Academy of Sciences USA* (1997) 94:7509-7514, which is hereby incorporated by reference in its entirety. Antibody homodimers can be converted to Fab'2 homodimers through digestion with pepsin. Alternatively, antibodies can be made to multimerize through recombinant DNA techniques. IgM and IgA naturally form antibody multimers through the interaction with the J chain polypeptide. Non-IgA or non-IgM molecules, such as IgG molecules, can be engineered to contain the J chain interaction domain of IgA or IgM, thereby conferring the ability to form higher order multimers on the non-IgA or non-IgM molecules. (see, for example, Chintalacharuvu et al., (2001) *Clinical Immunology* 101:21-31 and Frigerio et al., (2000) *Plant Physiology* 123:1483-94, both of which are hereby incorporated by reference in their entireties.) ScFv dimers can also be formed through recombinant techniques known in the art; an example of the construction of scFv dimers is given in Goel et al., (2000) *Cancer Research* 60:6964-6971, which is hereby incorporated by reference in its entirety. Antibody multimers may be purified using any suitable method known in the art, including, but not limited to, size exclusion chromatography.

[0034] The term "variant" as used herein refers to a polypeptide that possesses a similar or identical amino acid sequence as a PA polypeptide, a fragment of a PA polypeptide, an anti-PA antibody or antibody fragment thereof. A variant having a similar amino acid sequence refers to a polypeptide that satisfies at least one of the following: (a) a polypeptide comprising, or alternatively consisting of, an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of PA polypeptide (SEQ ID NO:2), a fragment of a PA polypeptide, an anti-PA antibody or antibody fragment thereof (including a VH domain, VHCDR, VL domain, or VLCDR having an amino acid sequence of any one or more scFvs or recombinant antibodies expressed by the cell lines in the ATCC Deposits referred to in Table 1) described herein; (b) a polypeptide encoded by a nucleotide sequence, the complementary sequence of which hybridizes under stringent conditions to a nucleotide sequence encoding PA (SEQ ID NO:2), a fragment of a PA polypeptide, an anti-PA antibody or antibody fragment thereof (including a VH domain, VHCDR, VL domain, or VLCDR having an amino acid sequence of any one of the scFvs referred to in Table 1), described herein, of at least 5 amino acid residues, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 30 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, or at least 150 amino acid residues; and (c) a polypeptide encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99%, identical to the nucleotide sequence encoding a PA polypeptide, a fragment of a PA polypeptide, an anti-PA antibody or antibody fragment thereof (including a VH domain, VHCDR, VL domain, or VLCDR having an amino acid sequence of any one or more scFvs or recombinant antibodies expressed by the cell lines in the ATCC Deposits referred to in Table 1), described herein. A polypeptide with similar structure to a PA polypeptide, a fragment of a PA polypeptide, an anti-PA antibody or antibody fragment thereof, described herein refers to a polypeptide that has a similar secondary, tertiary or quaternary structure of a PA polypeptide, a fragment of a PA

polypeptide, an anti-PA antibody, or antibody fragment thereof, described herein. The structure of a polypeptide can determined by methods known to those skilled in the art, including but not limited to, X-ray crystallography, nuclear magnetic resonance, and crystallographic electron microscopy. Preferably, a variant PA polypeptide, a variant fragment of a PA polypeptide, or a variant anti-PA antibody and/or antibody fragment possesses similar or identical function and/or structure as the reference PA polypeptide, the reference fragment of a PA polypeptide, or the reference anti-PA antibody and/or antibody fragment, respectively.

[0035] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide at the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

[0036] The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 87:2264-2268(1990), modified as in Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 90:5873-5877(1993). The BLASTn and BLASTx programs of Altschul, et al. *J. Mol. Biol.* 215:403-410(1990) have incorporated such an algorithm. BLAST nucleotide searches can be performed with the BLASTn program (score = 100, wordlength = 12) to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the BLASTx program (score = 50, wordlength = 3) to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. *Nucleic Acids Res.* 25:3589-3402(1997). Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*).

When utilizing BLAST, Gapped BLAST, and PSI-BLAST programs, the default parameters of the respective programs (e.g., BLASTx and BLASTn) can be used.

[0037] Another example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the GCG sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis and Robotti *Comput. Appl. Biosci., 10* :3-5(1994); and FASTA described in Pearson and Lipman *Proc. Natl. Acad. Sci. 85*:2444-8(1988). Within FASTA, ktup is a control option that sets the sensitivity and speed of the search.

[0038] The term "derivative" as used herein, refers to a variant polypeptide of the invention that comprises, or alternatively consists of, an amino acid sequence of a PA polypeptide, a fragment of a PA polypeptide, or an antibody of the invention that specifically binds to a PA polypeptide, which has been altered by the introduction of amino acid residue substitutions, deletions or additions. The term "derivative" as used herein also refers to a PA polypeptide, a fragment of a PA polypeptide, an antibody that specifically binds to a PA polypeptide which has been modified, *e.g.*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, a PA polypeptide, a fragment of a PA polypeptide, or an anti-PA antibody, may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a PA polypeptide, a fragment of a PA polypeptide, or an anti-PA antibody, may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a PA polypeptide, a fragment of a PA polypeptide, or an anti-PA antibody, may contain one or more non-classical amino acids. A polypeptide derivative possesses a similar or identical function as a PA polypeptide, a fragment of a PA polypeptide, or an anti-PA antibody, described herein.

[0039] The term "fragment" as used herein refers to a polypeptide comprising an amino acid sequence of at least 5 amino acid residues, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 30 amino acid residues, at least 35 amino acid residues, at least 40 amino

acid residues, at least 45 amino acid residues, at least 50 amino acid residues, at least 60 amino residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, at least 150 amino acid residues, at least 175 amino acid residues, at least 200 amino acid residues, or at least 250 amino acid residues, of the amino acid sequence of PA, or an anti-PA antibody (including molecules such as scFv's, that comprise, or alternatively consist of, antibody fragments or variants thereof) that specifically binds to PA.

[0040] The term "host cell" as used herein refers to the particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0041] Antibodies of the present invention are preferably provided in an isolated form, and preferably are substantially purified. By "isolated" is intended an antibody removed from its native environment. Thus, for example, an antibody produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention.

Antibody Structure

[0042] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kilodalton) and one "heavy" chain (about 50-70 kilodalton). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. Herein the terms "heavy chain" and "light chain" refer to the heavy and light chains of an antibody unless otherwise specified. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. *See generally, Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

[0043] Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

[0044] The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the heavy and the light chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987); Chothia et al., *Nature* 342:878-883 (1989).

[0045] A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. *See, e.g.,* Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. J *Immunol.* 148:1547 1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "'Diabodies': small bivalent and bispecific antibody fragments" PNAS USA 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).

[0046] Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are generally lower for bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

Anti-PA Antibodies

[0047] Using phage display technology, single chain antibody molecules ("scFvs") that specifically bind to PA (or fragments or variants thereof) have been identified (Example 1). Molecules comprising, or alternatively consisting of, fragments or variants of these scFvs (e.g., VH domains, VH CDRs, VL domains, or VL CDRs having an amino acid sequence of the corresponding region of the antibody expressed by a cell line contained in an ATCC Deposit referred to in Table 1), that specifically bind to PA (or

fragments or variants thereof) are also encompassed by the invention, as are nucleic acid molecules that encode these scFvs, and/or molecules.

[0048] In particular, the invention relates to scFvs comprising, or alternatively consisting of, an amino acid sequence selected from the group consisting of SEQ ID NOs: 48-56, preferably SEQ ID NOs:50 and 53 as referred to in Table 1 below. Molecules comprising, or alternatively consisting of, fragments or variants of these scFvs (e.g., VH domains, VH CDRs, VL domains, or VL CDRs having an amino acid sequence of any one of those referred to in Table 1), that specifically bind to PA are also encompassed by the invention, as are nucleic acid molecules that encode these scFvs, and/or molecules (e.g., SEQ ID NOs:57-65).

[0049] The present invention provides antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that specifically bind to a polypeptide or a polypeptide fragment of PA. In particular, the invention provides antibodies corresponding to the scFvs referred to in Table 1. Such scFvs may routinely be "converted" to immunoglobulin molecules by inserting, for example, the nucleotide sequences encoding the VH and/or VL domains of the scFv into an expression vector containing the constant domain sequences and engineered to direct the expression of the immunoglobulin molecule, as described in more detail in Example 6 below.

[0050] NS0 cell lines that express IgG1 antibodies that comprise the VH and VL domains of scFvs of the invention have been deposited with the American Type Culture Collection ("ATCC") on the dates listed in Table 1 and given the ATCC Deposit Numbers identified in Table 1. The ATCC is located at 10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

[0051] Accordingly, in one embodiment, the invention provides antibodies that comprise the VH and VL domains of scFvs of the invention.

[0052] In a preferred embodiment, an antibody of the invention is the antibody expressed by cell line NSO PA 2973 (PWD0587) #240-22 (See Table 1).

Table 1: Anti-PA scFvs

scFv	scFv protein SEQ ID NO:	scFv DNA SEQ ID NO:	AAs of VH Domain	AAs of VH CDR1	AAs of VH CDR2	AAs of VH CDR3	AAs of VL Domain	AAs of VL CDR1	AAs of VL CDR2	AAs of VL CDR3	Cell Line Expressing antibody	ATCC Deposit Number	ATCC Deposit Date
PWB2447	48	57	1-125	26-35	50-66	99-114	140-248	162-172	188-194	227-237			
PWC2004	49	58	1-123	26-35	50-66	99-112	140-251	162-175	191-197	230-240			
PWD0283	50	59	1-118	26-35	50-66	99-107	136-246	158-170	186-192	225-235			
PWD0323	51	60	1-117	26-35	50-66	99-106	134-244	156-168	184-190	223-233			
PWD0422	52	61	1-117	26-35	50-66	99-106	134-244	156-168	184-190	223-233			
PWD0587	53	62	1-117	26-35	50-66	99-106	134-244	156-168	184-190	223-233	NSO PA 2973 (PWD0587) #240-22	PTA-4796	November 11, 2002
PWD0791	54	63	1-120	26-35	50-66	99-109	138-248	160-172	188-194	227-237			
PHD2222	55	64	1-117	26-35	50-66	99-106	134-244	156-168	184-190	223-233			
PHD2581	56	65	1-117	26-35	50-66	99-106	134-244	156-168	184-190	223-233			

[0053] The present invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that specifically bind to a PA polypeptide or a fragment, variant, or fusion protein thereof. A PA polypeptide includes, but is not limited to, PA (SEQ ID NO:2).

Protective Antigen

[0054] Antibodies of the present invention bind PA polypeptide or fragments or variants thereof. The following section describes the PA polypeptides, fragments and variants that may be bound by the antibodies of the invention in more detail.

[0055] The PA protein is a 764 amino acid protein (SEQ ID NO:2) comprising a signal sequence from amino acid residues 1-29, and a 735 amino acid secreted protein which undergoes further process upon binding to an anthrax receptor, (e.g., ATR or CMG2) on the cell surface. The 735 amino acid secreted protein, also known as PA83 because it has a molecular weight of approximately 83 kilodaltons, has a structure that is largely made up of antiparallel beta pleated sheets with only a few short alpha-helices. The protein can be divided into four domains: Domain I (amino acid residues 30-287 of SEQ ID NO:2), Domain II (amino acid residues 288-516 of SEQ ID NO:2), Domain III (amino acid residues 517-624 of SEQ ID NO:2), and Domain IV (amino acid residues 625-764) of SEQ ID NO:2). In its native form, Domain I contains two calcium ions and the protease cleavage site RKKR at amino acid residues 193-196 of SEQ ID NO:2. Thus, Domain I contains the entire 20 kilodalton fragment (PA20, amino acid residues 30-196 of SEQ ID NO:2) that is cleaved off of PA upon binding to an anthrax receptor (e.g., ATR or CMG2) at the cell surface. That portion of Domain I that remains after cleavage of PA20 forms the N terminus of active PA63 and may be involved in binding LF and EF. Domain II is the heptamerization domain and also contains a large flexible loop that is implicated in membrane insertion. Domain III, is small and its function is not clearly understood. Domain IV is the receptor binding domain.

[0056] Thus, in specific embodiments, antibodies of the invention may bind the intact 735 amino acid secreted form of PA (PA83), polypeptides that comprise or alternatively consist of the PA63 protein, the PA20 fragment, and/or any one or more of domains I, II, III, or IV. In preferred embodiments, antibodies of the invention bind PA83 and prevent its cleavage of the PA20 fragment from the PA63 fragment by proteases. In other

embodiments, antibodies of the invention bind the PA63 form of PA and prevent oligomerization, and in particular heptamerization of PA63.

[0057] In certain embodiments, the antibodies of the present invention specifically bind PA polypeptide. An antibody that specifically binds PA may, in some embodiments, bind fragments, variants (including species orthologs of PA), multimers or modified forms of PA. For example, an antibody specific for PA may bind the PA moiety of a fusion protein comprising all or a portion of PA.

[0058] PA proteins may be found as monomers or multimers (i.e., dimers, trimers, tetramers, and higher multimers). Accordingly, the present invention relates to antibodies that bind PA proteins found as monomers or as part of multimers. In specific embodiments, antibodies of the invention bind PA monomers, dimers, trimers or heptamers. In additional embodiments, antibodies of the invention bind at least dimers, at least trimers, or at least tetramers containing one or more PA polypeptides.

[0059] Antibodies of the invention may bind PA homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only PA proteins of the invention (including PA fragments such as PA63, variants, and fusion proteins, as described herein). These homomers may contain PA proteins having identical or different polypeptide sequences. In a specific embodiment, a homomer of the invention is a multimer containing only PA proteins having an identical polypeptide sequence. In another specific embodiment, antibodies of the invention bind PA homomers containing PA proteins having different polypeptide sequences. In specific embodiments, antibodies of the invention bind a PA homodimer (e.g., containing PA proteins having identical or different polypeptide sequences). In additional embodiments, antibodies of the invention bind at least a homodimer, at least a homotrimer, or at least a homotetramer of PA.

[0060] In specific embodiments antibodies of the present invention bind PA homoheptamers.

[0061] As used herein, the term heteromer refers to a multimer containing heterologous proteins (i.e., proteins containing polypeptide sequences that do not correspond to a polypeptide sequences encoded by the PA gene) in addition to the PA proteins of the invention. In a specific embodiment, antibodies of the invention bind a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the

antibodies of the invention bind at least a heterodimer, at least a heterotrimer, or at least a heterotetramer containing one or more PA polypeptides.

[0062] In specific embodiments, antibodies of the present invention bind a PA heteroheptamer.

[0063] Antibodies of the invention may bind PA multimers that are the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, antibodies of the invention may bind PA multimers, such as, for example, homoheptamers, that are formed when PA proteins (such as PA63 polypeptide monomers) contact one another in solution. In another embodiment, antibodies of the invention may bind heteromultimers, such as, for example, heteroheptamers, that are formed when proteins of the invention contact antibodies to the PA polypeptides (including antibodies to the heterologous polypeptide sequence in a fusion protein) in solution. In other embodiments, multimers bound by one or more antibodies of the invention are formed by covalent associations with and/or between the PA proteins of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence of the protein (e.g., the polypeptide sequence recited in SEQ ID NO:2). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences of the proteins which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a PA fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a PA-Fc or PA-human serum albumin (PA-HSA) fusion protein (as described herein).

[0064] Antibodies of the invention may bind PA multimers generated using chemical techniques known in the art. For example, proteins desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers that antibodies of the invention may bind can be generated using techniques known in the art to form one or more inter-molecule cross-links between the

cysteine residues located within the polypeptide sequence of the proteins desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, proteins that antibodies of the invention may bind can be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide sequence of the protein and techniques known in the art may be applied to generate multimers containing one or more of these modified proteins (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the protein components desired to be contained in the multimer that antibodies of the invention may bind (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[0065] Alternatively, multimers that antibodies of the invention may bind can be generated using genetic engineering techniques known in the art. In one embodiment, proteins contained in multimers that may be bound by one or more antibodies of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer that may be bound by one or more antibodies of the invention are generated by ligating a polynucleotide sequence encoding a PA polypeptide to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant PA polypeptides which contain a transmembrane domain and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, two or more PA polypeptides are joined through synthetic linkers (e.g., peptide, carbohydrate or soluble polymer linkers). Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple PA polypeptides separated by peptide linkers may be produced using conventional recombinant DNA technology. In specific

embodiments, antibodies of the invention bind proteins comprising multiple PA polypeptides separated by peptide linkers.

[0066] Another method for preparing multimer PA polypeptides involves use of PA polypeptides fused to a leucine zipper or isoleucine polypeptide sequence. Leucine zipper domains and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric PA proteins are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a soluble PA polypeptide fused to a peptide that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric PA is recovered from the culture supernatant using techniques known in the art. In specific embodiments, antibodies of the invention bind PA-leucine zipper fusion protein monomers and/or PA-leucine zipper fusion protein multimers.

[0067] Antibodies that bind PA receptor polypeptides may bind them as isolated polypeptides or in their naturally occurring state. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example, a recombinantly produced version of the PA polypeptide may be substantially purified by the one-step method described in Smith and Johnson, *Gene 67*:31-40 (1988). Thus, antibodies of the present invention may bind recombinantly and/or naturally produced PA polypeptides. In a specific embodiment, antibodies of the present invention bind a PA secreted by a cell, preferably a bacterial cell, comprising a polynucleotide encoding amino acids 1 to 764 of SEQ ID NO:2 operably associated with a regulatory sequence that controls gene expression. In a specific embodiment, antibodies of the present invention bind PA purified from a bacterial cell culture, wherein said PA is encoded by a polynucleotide encoding amino acids 1 to 764 of SEQ ID NO:2 operably associated with a regulatory sequence that controls expression of said polynucleotide. In other specific embodiments, antibodies of the present invention bind a PA polypeptide expressed by a

cell comprising a polynucleotide encoding amino acids 197 to 764 of SEQ ID NO:2 operably associated with a regulatory sequence that controls gene expression. In still other embodiments, antibodies of the present invention bind a PA polypeptide expressed by a cell comprising a polynucleotide encoding amino acids 625 to 764 of SEQ ID NO:2 operably associated with a regulatory sequence that controls gene expression.

[0068] Antibodies of the present invention that may bind PA polypeptide fragments comprising or alternatively, consisting of, an amino acid sequence contained in SEQ ID NO:2. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Antibodies of the present invention may bind polypeptide fragments, including, for example, fragments that comprise or alternatively, consist of from about amino acid residues: 1 to 29, 30 to 59, 60 to 89, 90 to 119, 120 to149, 150 to 175, 176 to 196, 197 to 226, 227 to 256, 257 to 287, 288 to 312, 313 to 337, 338 to 362, 363 to 387, 388 to 412, 413 to 437, 438 to 462, 463 to 487, 488 to 516, 517 to 542, 543 to 569, 570 to 569, 570 to 596, 597 to 624, 625 to 652, 653 to 680, 681 to 708, 709 to 736, and/or 737 to 764 of SEQ ID NO:2. In this context "about" includes the particularly recited value, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Moreover, polypeptide fragments that antibodies of the invention may bind can be at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175 or 200 amino acids in length. In this context "about" includes the particularly recited value, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

[0069] Preferably, antibodies of the present invention bind polypeptide fragments selected from the group: a polypeptide comprising or alternatively, consisting of, the full length PA polypeptide (amino acid residues 1 to 764 in SEQ ID NO:2); a polypeptide comprising or alternatively, consisting of, the secreted form of PA (amino acid residues 30 to 764 in SEQ ID NO:2); a polypeptide comprising or alternatively, consisting of, the PA20 fragment (amino acid residues from about 30 to about 196 in SEQ ID NO:2); a polypeptide comprising or alternatively, consisting of, the PA63 fragment (amino acid residues from about 197 to about 764 in SEQ ID NO:2); a polypeptide comprising or alternatively, consisting of, PA domain I (amino acid residues 30 to 287 of SEQ ID NO:2); a polypeptide comprising or alternatively, consisting of, PA domain II (amino acid residues 288 to 516 of SEQ ID NO:2); a polypeptide comprising or alternatively,

consisting of, PA domain III (amino acid residues 517 to 624 of SEQ ID NO:2); a polypeptide comprising or alternatively, consisting of, PA domain IV (amino acid residues 625 to 764 of SEQ ID NO:2); a polypeptide comprising or alternatively, consisting of, fragment of the predicted mature PA polypeptide; and a polypeptide comprising, or alternatively, consisting of, one, two, three, four or more, epitope bearing portions of the PA receptor protein. In additional embodiments, the polypeptide fragments of the invention comprise, or alternatively, consist of, any combination of 1, 2, 3, 4, 5, 6, 7, or all 8 of the above members. The amino acid residues constituting these domains may vary slightly (e.g., by about 1 to about 15 amino acid residues) depending on the criteria used to define each domain.

[0070] Domain I contains the proteolytic cleavage site. When the secreted form of PA is cleaved at this site, a 20 kilodalton fragment (PA20) is released from PA, generating the biologically active 63 kilodalton PA63 fragment. Thus, in specific embodiments antibodies of the invention bind an epitope at or near this cleavage site and prevent the cleavage of the secreted form of PA that results in the generation of PA20 and PA63. In specific embodiments, antibodies of the invention that prevent cleavage of PA into PA20 and PA63 may bind one or more PA peptides (as well as the native amino acid secreted form of the protein, PA83, see, e.g., Example 2) selected from the group consisting of: (a) amino acid residues 190 to 209 of SEQ ID NO:2; (b) amino acid residues 181 to 201 of SEQ ID NO:2; (c) amino acid residues 198 to 212 of SEQ ID NO:2; (d) amino acid residues 196 to 212 of SEQ ID NO:2; (e) amino acid residues 194 to 212 of SEQ ID NO:2; (f) amino acid residues 192 to 212 of SEQ ID NO:2; (g) amino acid residues 190 to 212 of SEQ ID NO:2; (h) amino acid residues 188 to 212 of SEQ ID NO:2; (i) amino acid residues 186 to 212 of SEQ ID NO:2; (j) amino acid residues 184 to 212 of SEQ ID NO:2; and (k) amino acid residues 181 to 195 of SEQ ID NO:2.

[0071] Domain IV of PA is important for interactions between PA and its receptor (e.g., ATR (SEQ ID NO:3) or CMG2 (SEQ ID NO:42)). Accordingly, in preferred embodiments, antibodies of the present invention bind PA polypeptide fragments comprising, or alternatively consisting of amino acid residues 625 to 764 of SEQ ID NO:2. In preferred embodiments, the antibodies of the invention that bind all or a portion of domain IV of PA prevent PA from binding to ATR and/or CMG2. In other preferred embodiments, the antibodies of the invention that bind all or a portion of domain IV of PA protect cells from death induced by anthrax toxins.

[0072] Antibodies of the invention may also bind fragments comprising, or alternatively, consisting of structural or functional attributes of PA. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) PA. Certain preferred regions are those set out in Table 2 and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in (SEQ ID NO:2), such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle predicted hydrophilic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs.

[0073] The data representing the structural or functional attributes of PA set forth in Table 2, as described above, was generated using the various modules and algorithms of the DNA*STAR set on default parameters. Column I represents the results of a Garnier-Robson analysis of alpha helical regions; Column II represents the results of a Chou-Fasman analysis of alpha helical regions; Column III represents the results of a Garnier Robson analysis of beta sheet regions; Column IV represents the results of a Chou-Fasman analysis of beta sheet regions; Column V represents the results of a Garnier Robson analysis of turn regions; Column VI represents the results of a Chou-Fasman analysis of turn regions; Column VII represents the results of a Garnier Robson analysis of coil regions; Column VIII represents a Kyte-Doolittle hydrophilicity plot; Column; Column IX represents the results of an Eisenberg analysis of alpha amphipathic regions; Column X represents the results of an Eisenberg analysis of beta amphipathic regions; Column XI represents the results of a Karplus-Schultz analysis of flexible regions; Column XII represents the Jameson-Wolf antigenic index score; and Column XIII represents the Emini surface probability plot.

[0074] In a preferred embodiment, the data presented in columns VIII, XII, and XIII of Table 2 can be used to determine regions of PA which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, XII, and/or XIII by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

[0075] The above-mentioned preferred regions set out in Table 2 include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in SEQ ID NO:2. As set out in Table 2, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Jameson-Wolf regions of high antigenic index and Emini surface-forming regions. Among preferred polypeptide fragments bound by one or more antibodies of the invention are those that comprise regions of PA that combine several structural features, such as several (e.g., 1, 2, 3, or 4) of the same or different region features set out above and in Table 2.

Table 2

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Met	1	A	A	.	.	.	.	.	1.59	.	.	.	0.75	2.90
Lys	2	A	A	.	.	.	.	.	1.12	.	.	.	0.75	4.54
Lys	3	A	A	.	B	.	.	.	0.70	.	.	.	0.75	2.64
Arg	4	A	A	.	B	.	.	.	0.20	.	.	.	0.75	2.20
Lys	5	A	A	.	B	.	.	.	0.38	.	.	F	0.75	0.77
Val	6	.	A	B	B	.	.	.	0.17	.	.	.	0.60	0.60
Leu	7	.	A	B	B	.	.	.	-0.48	.	.	.	-0.30	0.25
Ile	8	.	A	B	B	.	.	.	-1.11	.	.	.	-0.60	0.12
Pro	9	.	A	B	B	.	.	.	-2.03	*	.	.	-0.60	0.17
Leu	10	.	A	B	B	.	.	.	-2.38	.	*	.	-0.60	0.17
Met	11	A	A	.	B	.	.	.	-1.83	.	.	.	-0.60	0.32
Ala	12	A	A	.	B	.	.	.	-1.91	.	.	.	-0.60	0.30
Leu	13	A	A	.	B	.	.	.	-1.83	.	.	.	-0.60	0.26
Ser	14	.	A	B	B	.	.	.	-2.48	.	.	.	-0.60	0.21
Thr	15	.	.	B	B	.	.	.	-1.97	.	.	.	-0.60	0.16
Ile	16	.	.	B	B	.	.	.	-1.67	.	.	.	-0.60	0.25
Leu	17	.	.	B	B	.	.	.	-1.39	.	.	.	-0.60	0.25
Val	18	.	.	B	B	.	.	.	-0.92	.	.	.	-0.60	0.25
Ser	19	.	.	B	B	.	.	.	-0.62	.	*	F	-0.36	0.36
Ser	20	.	.	.	.	.	T	C	-1.12	.	*	F	0.33	0.70
Thr	21	.	.	.	.	.	T	C	-0.23	.	*	F	0.42	0.78
Gly	22	.	.	.	.	.	T	C	-0.28	.	*	F	1.56	1.01
Asn	23	.	.	.	.	.	T	C	-0.31	.	*	F	0.90	0.56
Leu	24	A	A	.	.	.	.	.	-0.01	.	.	.	0.06	0.27
Glu	25	A	A	.	.	.	.	.	-0.30	.	.	.	-0.03	0.47
Val	26	A	A	.	.	.	.	.	0.01	.	*	.	-0.12	0.30
Ile	27	A	A	.	.	.	.	.	-0.50	.	*	.	0.39	0.63
Gln	28	A	A	.	.	.	.	.	-0.46	*	*	.	0.30	0.27
Ala	29	A	A	.	.	.	.	.	0.36	*	*	.	0.30	0.72
Glu	30	A	A	.	.	.	.	.	0.36	*	*	.	0.45	1.79
Val	31	A	A	.	.	.	.	.	1.21	*	*	F	0.90	1.79
Lys	32	A	A	.	.	.	.	.	2.21	*	*	F	0.90	2.84
Gln	33	A	A	.	.	.	.	.	1.40	*	*	F	0.90	3.21
Glu	34	A	A	.	.	.	.	.	1.18	*	*	F	0.90	3.57
Asn	35	A	A	.	.	.	.	.	1.18	*	*	F	0.90	1.47
Arg	36	A	A	.	.	.	.	.	2.03	*	*	F	0.60	1.37
Leu	37	A	A	.	.	.	.	.	1.69	*	.	F	0.90	1.37
Leu	38	A	A	.	.	.	.	.	1.69	*	.	F	0.94	1.14
Asn	39	.	.	.	.	.	T	C	1.39	*	.	F	2.18	1.01
Glu	40	A	.	.	.	.	T	.	1.09	*	.	F	2.02	1.64
Ser	41	.	.	.	.	.	T	C	0.68	*	.	F	2.86	2.66
Glu	42	.	.	.	.	T	T	.	1.49	.	.	F	3.40	2.22
Ser	43	.	.	.	.	T	T	.	1.96	.	.	F	3.06	2.22
Ser	44	.	.	.	.	T	T	.	1.14	.	.	F	2.72	1.64
Ser	45	.	.	.	.	T	T	.	0.33	.	.	F	1.93	0.78
Gln	46	.	.	B	.	.	T	.	0.29	.	.	F	0.59	0.48
Gly	47	.	.	B	B	.	.	.	0.04	.	.	F	-0.45	0.35
Leu	48	.	.	B	B	.	.	.	0.10	.	.	F	-0.45	0.41
Leu	49	.	.	B	B	.	.	.	-0.30	.	.	.	-0.60	0.37
Gly	50	.	.	B	B	.	.	.	-0.30	.	.	.	-0.60	0.33
Tyr	51	.	.	B	B	.	.	.	-0.30	.	.	.	-0.60	0.53
Tyr	52	.	.	B	B	.	.	.	-0.77	.	.	.	-0.45	1.08
Phe	53	.	.	B	B	.	.	.	0.04	.	.	.	-0.60	0.90
Ser	54	.	.	B	.	.	.	.	0.16	.	*	.	-0.40	0.92
Asp	55	.	A	B	.	.	.	.	0.50	.	*	.	-0.60	0.51
Leu	56	.	A	B	.	.	.	.	0.16	.	*	.	-0.45	1.02
Asn	57	.	A	.	.	T	.	.	0.19	.	*	.	0.10	0.77
Phe	58	.	A	.	.	T	.	.	0.29	.	*	.	0.10	0.71
Gln	59	A	A	.	.	.	.	.	-0.27	.	*	.	-0.60	0.85
Ala	60	.	A	B	B	.	.	.	-1.12	.	*	.	-0.60	0.39

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Pro	61	.	A	B	B	.	.	.	-0.62	.	*	.	-0.60	0.34
Met	62	.	A	B	B	.	.	.	-0.92	.	*	.	-0.60	0.28
Val	63	.	A	B	B	.	.	.	-0.52	.	.	.	-0.60	0.37
Val	64	.	.	B	B	.	.	.	-0.83	.	.	.	-0.60	0.32
Thr	65	.	.	B	B	.	.	.	-0.56	.	.	F	-0.20	0.47
Ser	66	.	.	B	B	.	.	.	-0.69	.	.	F	0.05	0.92
Ser	67	.	.	B	B	.	.	.	-0.09	.	.	F	0.75	1.22
Thr	68	.	.	B	.	.	T	.	-0.04	.	.	F	2.00	1.42
Thr	69	.	.	.	.	T	T	.	0.51	.	*	F	2.50	0.87
Gly	70	.	.	.	.	T	T	.	-0.07	.	*	F	2.25	0.87
Asp	71	.	.	B	.	.	T	.	0.02	.	*	F	1.00	0.42
Leu	72	.	.	B	.	.	.	.	0.02	.	*	F	0.55	0.45
Ser	73	.	.	B	.	.	.	.	0.03	.	*	F	0.90	0.61
Ile	74	.	.	B	.	.	T	.	0.34	.	*	F	0.85	0.49
Pro	75	.	.	B	.	.	T	.	-0.12	.	*	F	1.00	1.04
Ser	76	.	.	.	.	.	T	C	-0.12	.	.	F	1.05	0.64
Ser	77	.	.	.	.	.	T	C	0.69	*	.	F	1.20	1.57
Glu	78	A	A	.	.	.	.	.	0.10	*	.	F	0.90	1.64
Leu	79	.	A	B	.	.	.	.	0.78	*	.	F	0.71	0.86
Glu	80	.	A	.	.	T	.	.	0.69	*	.	F	1.37	0.99
Asn	81	.	A	.	.	.	.	C	0.99	.	.	F	1.43	0.76
Ile	82	.	.	.	.	.	.	C	1.29	*	.	F	2.04	1.61
Pro	83	.	.	.	.	.	.	C	1.29	.	.	F	2.60	1.49
Ser	84	.	.	.	.	T	T	.	1.86	*	.	F	2.44	1.61
Glu	85	A	.	.	.	.	T	.	1.16	*	.	F	1.18	3.59
Asn	86	A	.	.	.	.	T	.	1.16	.	.	F	0.92	2.01
Gln	87	.	.	.	.	T	T	.	1.74	*	.	F	1.06	2.60
Tyr	88	.	.	B	B	.	.	.	1.37	*	.	.	-0.15	2.01
Phe	89	.	.	B	B	.	.	.	0.78	.	.	.	-0.45	1.26
Gln	90	.	.	B	B	.	.	.	0.49	.	.	.	-0.60	0.51
Ser	91	.	.	B	B	.	.	.	0.19	*	.	.	-0.60	0.34
Ala	92	.	.	B	B	.	.	.	-0.16	*	.	.	-0.60	0.53
Ile	93	.	.	B	B	.	.	.	-0.61	.	.	.	-0.60	0.30
Trp	94	A	.	.	.	.	T	.	-0.80	*	*	.	-0.20	0.20
Ser	95	A	.	.	.	.	T	.	-0.76	*	*	.	-0.20	0.14
Gly	96	A	.	.	.	.	T	.	-1.31	*	*	.	-0.20	0.39
Phe	97	A	.	.	.	.	T	.	-0.68	*	*	.	-0.20	0.27
Ile	98	.	A	B	B	.	.	.	0.26	*	*	.	0.30	0.41
Lys	99	A	A	.	B	.	.	.	0.24	.	*	.	0.60	0.83
Val	100	.	A	.	B	.	.	C	0.54	.	*	F	1.70	1.28
Lys	101	.	A	.	B	.	.	C	0.89	.	*	F	2.00	3.05
Lys	102	.	A	.	.	.	.	C	1.34	.	.	F	2.30	2.64
Ser	103	.	.	.	.	.	T	C	1.92	.	*	F	3.00	5.57
Asp	104	A	.	.	.	.	T	.	1.18	.	.	F	2.50	4.02
Glu	105	A	.	.	.	.	T	.	1.44	.	.	F	2.20	1.74
Tyr	106	.	.	B	.	.	T	.	1.09	.	.	.	1.45	1.31
Thr	107	A	.	.	B	.	.	.	0.74	.	.	.	0.15	1.13
Phe	108	A	.	.	B	.	.	.	0.46	.	.	.	-0.30	0.88
Ala	109	A	.	.	B	.	.	.	0.46	.	.	.	-0.60	0.57
Thr	110	A	.	.	B	.	.	.	0.46	.	.	F	0.06	0.65
Ser	111	A	.	.	.	.	T	.	0.67	*	.	F	0.82	1.22
Ala	112	A	.	.	.	.	T	.	0.12	*	.	F	1.63	1.64
Asp	113	A	.	.	.	.	T	.	0.51	*	.	F	1.69	0.84
Asn	114	.	.	.	.	.	T	C	0.50	*	.	F	2.10	0.91
His	115	.	.	.	B	.	.	C	0.52	*	.	.	0.74	0.89
Val	116	.	.	B	B	.	.	.	-0.03	*	*	.	0.03	0.56
Thr	117	.	.	B	B	.	.	.	0.56	*	.	.	-0.18	0.26
Met	118	.	.	B	B	.	.	.	0.56	.	.	.	-0.39	0.32
Trp	119	A	.	.	B	.	.	.	0.56	.	.	.	-0.30	0.71
Val	120	A	.	.	.	.	T	.	0.59	.	.	.	0.10	0.86

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Asp	121	A	.	.	.	.	T	.	0.59	.	.	F	1.00	1.50
Asp	122	A	.	.	.	.	T	.	0.01	.	.	F	1.00	1.06
Gln	123	A	.	.	.	.	T	.	0.61	*	.	F	1.15	1.00
Glu	124	A	A	.	B	.	.	.	0.94	*	.	F	0.75	0.96
Val	125	A	A	.	B	.	.	.	1.21	.	.	.	0.75	1.15
Ile	126	A	A	.	B	.	.	.	0.91	*	.	.	0.30	0.67
Asn	127	A	A	.	B	.	.	.	0.91	*	.	.	0.60	0.52
Lys	128	A	A	.	.	.	.	.	0.61	*	.	F	0.60	1.13
Ala	129	A	A	.	.	.	.	.	0.61	*	.	F	1.50	2.15
Ser	130	.	A	.	.	.	.	C	1.51	*	.	F	2.30	2.15
Asn	131	.	.	.	.	.	T	C	1.51	.	*	F	3.00	2.15
Ser	132	.	.	.	.	.	T	C	1.62	.	*	F	2.40	1.49
Asn	133	A	.	.	.	.	T	.	0.77	.	*	F	2.20	2.18
Lys	134	.	.	B	.	.	T	.	1.36	.	*	F	1.60	1.12
Ile	135	.	A	B	.	.	.	.	1.70	.	*	F	1.20	1.45
Arg	136	.	A	B	.	.	.	.	1.36	.	*	.	0.75	1.80
Leu	137	.	A	B	.	.	.	.	1.77	.	*	F	0.75	0.89
Glu	138	A	A	.	.	.	.	.	0.96	.	*	F	0.90	2.49
Lys	139	A	A	.	.	.	.	.	0.67	.	*	F	0.90	1.05
Gly	140	A	.	.	B	.	.	.	1.56	*	.	F	0.60	1.99
Arg	141	A	.	.	B	.	.	.	0.56	.	.	F	0.90	1.99
Leu	142	A	.	.	B	.	.	.	1.41	.	*	.	0.30	0.70
Tyr	143	A	.	.	B	.	.	.	0.52	.	*	.	0.45	1.41
Gln	144	.	.	B	B	.	.	.	0.48	.	*	.	-0.30	0.50
Ile	145	.	.	B	B	.	.	.	0.58	.	*	.	-0.45	1.06
Lys	146	.	.	B	B	.	.	.	0.47	*	*	.	-0.45	1.06
Ile	147	.	.	B	B	.	.	.	1.39	*	*	.	-0.15	1.06
Gln	148	.	.	B	B	.	.	.	1.63	.	*	.	0.45	2.96
Tyr	149	.	.	B	.	.	.	.	1.63	.	*	.	0.95	2.56
Gln	150	.	.	B	.	.	.	.	2.31	.	*	F	0.80	5.88
Arg	151	.	.	.	.	T	.	.	1.96	.	.	F	1.84	5.25
Glu	152	.	.	.	.	.	.	C	2.84	.	.	F	1.98	4.83
Asn	153	.	.	.	.	.	T	C	2.89	.	.	F	2.52	4.83
Pro	154	.	.	.	.	.	T	C	2.79	.	.	F	2.86	4.93
Thr	155	.	.	.	.	T	T	.	1.98	.	.	F	3.40	2.82
Glu	156	A	.	.	.	.	T	.	1.87	.	*	F	2.66	1.45
Lys	157	A	A	.	.	.	.	.	1.17	.	*	F	1.92	1.56
Gly	158	A	A	.	.	.	.	.	1.21	.	*	F	1.43	0.94
Leu	159	A	A	.	.	.	.	.	0.61	.	*	.	1.09	1.08
Asp	160	A	A	.	.	.	.	.	0.68	.	*	.	0.30	0.45
Phe	161	.	.	B	B	.	.	.	0.39	.	*	.	-0.30	0.71
Lys	162	.	.	B	B	.	.	.	0.03	.	*	.	-0.60	0.90
Leu	163	.	.	B	B	.	.	.	0.38	.	*	.	-0.60	0.78
Tyr	164	.	.	B	B	.	.	.	0.89	.	*	.	-0.45	1.50
Trp	165	A	.	.	B	.	.	.	0.89	.	*	.	0.15	1.01
Thr	166	A	.	.	B	.	.	.	1.59	.	*	F	0.30	2.11
Asp	167	A	.	.	B	.	.	.	1.59	.	.	F	0.90	2.17
Ser	168	A	.	.	.	.	T	.	2.44	.	.	F	2.20	4.12
Gln	169	.	.	.	.	.	T	C	2.69	.	.	F	3.00	5.71
Asn	170	.	.	.	.	.	T	C	2.12	.	.	F	2.70	5.93
Lys	171	.	.	.	.	.	T	C	1.54	.	.	F	2.40	3.28
Lys	172	.	A	B	.	.	.	.	1.24	.	.	F	1.50	1.33
Glu	173	.	A	B	.	.	.	.	1.24	.	.	F	1.20	1.11
Val	174	.	A	B	.	.	.	.	1.24	.	.	F	1.03	0.74
Ile	175	.	A	B	.	.	.	.	1.24	.	.	F	1.31	0.62
Ser	176	.	.	B	.	.	T	.	0.39	.	.	F	1.99	0.58
Ser	177	.	.	B	.	.	T	.	0.34	.	.	F	1.37	0.64
Asp	178	.	.	.	.	T	T	.	-0.47	.	.	F	2.80	1.58
Asn	179	.	.	.	.	.	T	C	0.18	.	.	F	2.17	0.97
Leu	180	A	A	.	.	.	.	.	1.07	.	.	.	1.29	1.12

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Gln	181	A	A	.	.	.	.	.	0.56	.	.	.	1.01	1.16
Leu	182	A	A	.	.	.	.	.	0.90	.	.	.	-0.02	0.60
Pro	183	A	A	.	.	.	.	.	0.90	.	.	F	0.60	1.45
Glu	184	A	A	.	.	.	.	.	0.94	.	*	F	0.60	1.45
Leu	185	A	A	.	.	.	.	.	1.46	.	.	F	0.90	3.51
Lys	186	A	A	.	.	.	.	.	1.16	*	.	F	0.90	3.04
Gln	187	A	A	.	.	.	.	.	1.97	*	.	F	1.24	2.35
Lys	188	A	A	.	.	.	.	.	1.88	.	*	F	1.58	4.59
Ser	189	A	.	.	.	.	T	.	1.99	*	*	F	2.32	3.07
Ser	190	A	.	.	.	.	T	.	2.84	.	*	F	2.66	3.48
Asn	191	.	.	.	.	T	T	.	2.84	.	.	F	3.40	3.48
Ser	192	.	.	.	.	T	T	.	2.96	.	.	F	3.06	5.19
Arg	193	.	.	.	.	T	.	.	2.61	.	.	F	2.52	7.58
Lys	194	.	.	.	.	T	.	.	2.60	.	.	F	2.18	6.32
Lys	195	.	.	.	.	T	.	.	2.60	.	.	F	1.84	6.80
Arg	196	.	.	B	.	.	.	.	2.01	.	.	F	1.10	4.65
Ser	197	.	.	B	.	.	.	.	1.97	.	.	F	1.10	2.35
Thr	198	.	.	B	.	.	.	.	1.64	.	.	F	1.10	1.16
Ser	199	.	.	.	.	T	T	.	1.29	.	.	F	1.25	0.92
Ala	200	.	.	.	.	.	T	C	0.39	*	.	F	0.71	0.99
Gly	201	.	.	.	.	.	T	C	0.07	*	.	F	0.97	0.51
Pro	202	.	.	B	.	.	T	.	0.37	*	.	F	1.03	0.59
Thr	203	.	.	B	.	.	.	.	0.79	.	.	F	1.69	0.97
Val	204	.	.	B	.	.	T	.	1.09	.	.	F	2.60	1.92
Pro	205	.	.	B	.	.	T	.	1.68	*	.	F	2.34	2.07
Asp	206	.	.	B	.	.	T	.	2.02	*	.	F	2.42	2.31
Arg	207	.	.	B	.	.	T	.	1.89	*	.	F	2.50	5.20
Asp	208	.	.	.	.	T	T	.	1.31	*	.	F	2.98	3.33
Asn	209	.	.	.	.	T	T	.	1.96	*	.	F	3.06	1.40
Asp	210	.	.	.	.	T	T	.	2.17	*	.	F	3.40	1.10
Gly	211	.	.	.	.	.	T	C	1.87	*	.	F	2.86	1.10
Ile	212	.	.	.	.	.	T	C	0.94	*	.	F	2.37	0.92
Pro	213	.	.	.	.	.	T	C	0.94	*	.	F	1.73	0.45
Asp	214	.	.	.	.	.	T	C	0.09	*	*	F	1.39	0.79
Ser	215	.	.	B	.	.	T	.	0.09	*	*	F	0.85	0.84
Leu	216	.	A	B	.	.	.	.	0.09	.	*	.	0.60	0.94
Glu	217	.	A	B	.	.	.	.	0.73	.	*	.	0.60	0.56
Val	218	A	A	.	.	.	.	.	0.63	.	.	.	0.30	0.65
Glu	219	A	A	.	.	.	.	.	-0.22	.	.	.	0.45	1.14
Gly	220	A	A	.	.	.	.	.	0.08	.	*	.	0.30	0.49
Tyr	221	A	.	.	B	.	.	.	0.03	.	*	.	0.45	1.10
Thr	222	A	.	.	B	.	.	.	0.08	.	*	.	0.30	0.47
Val	223	A	.	.	B	.	.	.	0.93	.	*	.	0.56	0.95
Asp	224	A	.	.	B	.	.	.	0.98	.	.	.	0.82	0.98
Val	225	A	.	.	.	.	.	.	1.43	.	*	F	1.88	1.36
Lys	226	A	.	.	.	.	.	.	1.37	.	*	F	2.14	3.58
Asn	227	.	.	B	.	.	T	.	0.98	.	*	F	2.60	3.09
Lys	228	.	.	B	.	.	T	.	1.02	.	*	F	2.34	3.61
Arg	229	.	.	B	.	.	T	.	0.72	.	.	F	2.08	1.49
Thr	230	.	.	B	.	.	T	.	1.37	*	.	F	1.52	1.24
Phe	231	.	.	B	.	.	.	.	1.03	*	.	F	0.91	0.96
Leu	232	.	.	B	.	.	.	.	0.14	.	.	.	-0.40	0.51
Ser	233	.	.	.	.	.	T	C	-0.20	.	.	.	0.00	0.25
Pro	234	.	.	.	.	T	T	.	-0.31	*	.	.	0.20	0.39
Trp	235	.	.	.	.	T	T	.	-0.89	*	.	.	0.20	0.75
Ile	236	A	.	.	.	.	T	.	-0.22	*	.	.	-0.20	0.39
Ser	237	A	.	.	B	.	.	.	0.59	*	.	.	-0.60	0.35
Asn	238	A	A	.	B	.	.	.	0.93	.	.	.	-0.60	0.57
Ile	239	A	A	.	B	.	.	.	1.19	.	.	.	0.45	1.63
His	240	A	A	.	B	.	.	.	1.13	.	.	.	0.75	2.44

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Glu	241	A	A	.	.	.	.	.	1.21	.	.	F	0.90	1.50
Lys	242	A	A	.	.	.	.	.	1.20	.	.	F	0.90	1.76
Lys	243	A	A	.	.	.	.	.	1.24	.	.	F	0.90	1.87
Gly	244	A	A	.	.	.	.	.	1.89	.	*	F	0.90	2.16
Leu	245	A	.	.	.	.	.	.	1.97	*	.	F	1.44	1.69
Thr	246	A	.	.	.	.	T	.	1.67	*	.	F	1.98	1.69
Lys	247	.	.	B	.	.	T	.	1.32	*	.	F	2.02	2.29
Tyr	248	.	.	B	.	.	T	.	1.07	*	*	F	2.36	3.72
Lys	249	.	.	.	.	T	T	.	1.41	*	.	F	3.40	3.99
Ser	250	.	.	.	.	.	.	C	2.27	*	.	F	2.66	3.45
Ser	251	.	.	.	.	.	T	C	2.29	*	*	F	2.52	4.41
Pro	252	.	.	.	.	.	T	C	1.94	*	*	F	2.18	2.32
Glu	253	.	.	.	.	T	T	.	1.88	*	.	F	2.04	2.32
Lys	254	.	.	.	.	T	T	.	1.24	*	.	F	1.40	2.50
Trp	255	.	.	.	.	T	.	.	1.24	.	.	F	1.20	1.63
Ser	256	.	.	B	.	.	.	.	1.54	.	.	F	0.80	1.26
Thr	257	.	.	B	.	.	.	.	1.54	*	.	F	1.10	1.05
Ala	258	.	.	.	.	T	.	.	1.30	*	.	F	1.20	1.55
Ser	259	.	.	.	.	.	.	C	0.96	*	.	F	1.90	1.81
Asp	260	.	.	.	.	.	T	C	1.24	*	.	F	2.40	1.68
Pro	261	.	.	.	.	.	T	C	0.84	*	.	F	3.00	2.78
Tyr	262	.	.	.	.	T	T	.	1.16	*	.	F	2.60	1.80
Ser	263	.	.	.	.	.	T	C	1.79	*	.	F	2.40	1.86
Asp	264	A	A	.	.	.	.	.	1.23	*	.	F	1.50	2.41
Phe	265	A	A	.	.	.	.	.	0.92	*	.	F	0.90	1.14
Glu	266	A	A	.	.	.	.	.	0.79	*	*	F	0.90	1.23
Lys	267	A	A	.	.	.	.	.	1.14	*	*	F	0.75	0.73
Val	268	A	A	.	.	.	.	.	0.56	*	*	F	0.90	1.65
Thr	269	A	.	.	B	.	.	.	0.56	*	*	F	0.75	0.67
Gly	270	A	.	.	B	.	.	.	1.30	*	*	F	0.75	0.56
Arg	271	A	.	.	B	.	.	.	1.30	*	*	F	0.90	1.50
Ile	272	.	.	B	B	.	.	.	0.40	*	*	F	1.20	1.67
Asp	273	.	.	.	.	T	T	.	0.96	*	*	F	2.30	1.25
Lys	274	.	.	.	.	.	T	C	1.06	*	*	F	2.25	0.86
Asn	275	.	.	.	.	.	T	C	1.40	*	*	F	2.40	1.89
Val	276	.	.	.	.	.	T	C	0.70	*	*	F	3.00	1.96
Ser	277	.	.	.	.	.	T	C	1.70	*	*	F	2.55	0.99
Pro	278	.	.	.	.	.	T	C	1.67	*	*	F	2.40	1.21
Glu	279	A	.	.	.	.	T	.	1.41	*	*	F	1.90	2.21
Ala	280	A	.	.	.	.	T	.	0.60	*	*	F	1.60	2.56
Arg	281	A	.	.	.	.	.	.	0.60	.	*	.	0.65	1.36
His	282	.	.	B	B	.	.	.	0.31	.	*	.	0.30	0.58
Pro	283	.	.	B	B	.	.	.	-0.07	.	*	.	-0.30	0.58
Leu	284	A	.	.	B	.	.	.	-0.31	.	*	.	-0.30	0.30
Val	285	A	.	.	B	.	.	.	0.07	*	*	.	-0.60	0.35
Ala	286	A	.	.	B	.	.	.	-0.93	*	.	.	-0.60	0.35
Ala	287	.	.	B	B	.	.	.	-1.76	.	.	.	-0.60	0.29
Tyr	288	.	.	B	B	.	.	.	-1.58	.	.	.	-0.60	0.29
Pro	289	.	.	B	B	.	.	.	-1.62	.	.	.	-0.60	0.40
Ile	290	.	.	B	B	.	.	.	-0.77	.	.	.	-0.60	0.29
Val	291	.	.	B	B	.	.	.	-0.78	.	*	.	-0.60	0.31
His	292	.	.	B	B	.	.	.	-0.19	.	*	.	-0.60	0.20
Val	293	.	.	B	B	.	.	.	0.06	.	.	.	-0.30	0.49
Asp	294	A	.	.	B	.	.	.	-0.62	.	.	.	0.45	1.07
Met	295	A	A	.	.	.	.	.	-0.62	.	.	.	0.30	0.55
Glu	296	A	A	.	.	.	.	.	-0.58	.	*	.	-0.30	0.52
Asn	297	A	A	.	.	.	.	.	-0.84	.	.	.	-0.30	0.26
Ile	298	A	A	.	.	.	.	.	0.06	.	.	.	-0.60	0.35
Ile	299	A	A	.	.	.	.	.	0.06	.	.	.	0.30	0.40

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Leu	300	A	A	.	.	.	.	.	0.66	.	.	.	0.04	0.40
Ser	301	A	A	.	.	.	.	.	0.66	.	.	F	1.13	0.99
Lys	302	A	A	.	.	.	.	.	0.66	.	.	F	1.92	2.36
Asn	303	.	.	.	.	.	T	C	1.24	.	.	F	2.86	4.95
Glu	304	.	.	.	.	T	T	.	1.82	*	.	F	3.40	4.95
Asp	305	.	.	.	.	T	T	.	2.63	*	.	F	3.06	3.57
Gln	306	.	.	.	.	T	T	.	2.93	.	.	F	2.72	3.85
Ser	307	.	.	.	.	T	.	.	2.58	.	.	F	2.18	3.57
Thr	308	.	.	B	.	.	.	.	2.58	.	.	F	1.42	3.09
Gln	309	.	.	.	.	T	.	.	2.28	.	.	F	1.76	2.98
Asn	310	.	.	.	.	.	T	C	2.28	.	.	F	2.04	2.98
Thr	311	.	.	.	.	.	T	C	1.97	*	*	F	2.32	3.57
Asp	312	.	.	.	.	T	T	.	2.38	.	*	F	2.80	2.98
Ser	313	.	.	B	.	.	T	.	2.38	*	.	F	2.42	3.62
Gln	314	.	.	B	B	.	.	.	1.49	*	.	F	1.74	3.62
Thr	315	.	.	B	B	.	.	.	1.19	*	*	F	1.46	1.52
Arg	316	.	.	B	B	.	.	.	1.54	*	.	F	0.88	1.52
Thr	317	.	.	B	B	.	.	.	1.54	*	.	F	0.90	1.76
Ile	318	.	.	B	B	.	.	.	1.53	*	.	F	0.90	1.96
Ser	319	.	.	B	.	.	T	.	1.23	*	.	F	1.60	1.44
Lys	320	.	.	.	.	T	T	.	1.23	*	.	F	2.00	1.34
Asn	321	.	.	.	.	.	T	C	0.82	*	.	F	2.10	2.76
Thr	322	.	.	.	.	.	T	C	1.24	.	.	F	2.40	2.76
Ser	323	.	.	.	.	.	T	C	1.82	.	.	F	3.00	2.70
Thr	324	.	.	.	.	.	T	C	2.09	.	.	F	2.40	2.42
Ser	325	.	.	.	.	.	T	C	1.73	.	.	F	2.36	2.29
Arg	326	.	.	.	.	.	T	C	1.43	.	.	F	2.32	2.46
Thr	327	.	.	.	.	.	.	C	1.74	.	.	F	2.08	2.29
His	328	.	.	.	.	.	T	C	1.19	*	.	F	2.54	2.95
Thr	329	.	.	B	.	.	T	.	1.47	.	.	F	2.60	1.12
Ser	330	.	.	B	.	.	T	.	1.42	.	*	F	2.04	1.06
Glu	331	.	.	B	.	.	T	.	1.31	.	*	F	1.63	0.77
Val	332	.	.	.	.	.	.	C	1.03	.	*	F	1.37	0.86
His	333	.	.	.	.	.	T	C	1.07	.	*	F	1.31	0.64
Gly	334	.	.	.	.	.	T	C	0.52	.	*	.	1.20	0.64
Asn	335	A	.	.	.	.	T	.	0.79	.	*	.	0.10	0.64
Ala	336	A	.	.	.	.	T	.	0.20	.	*	.	0.70	0.64
Glu	337	A	A	.	.	.	.	.	0.76	.	*	.	0.30	0.66
Val	338	A	A	.	.	.	.	.	0.09	.	*	.	0.30	0.55
His	339	A	A	.	.	.	.	.	-0.27	.	*	.	-0.30	0.47
Ala	340	A	A	.	.	.	.	.	-0.27	.	*	.	-0.60	0.24
Ser	341	.	A	B	.	.	.	.	-0.57	*	*	.	-0.60	0.53
Phe	342	.	A	B	.	.	.	.	-0.91	*	.	.	-0.60	0.27
Phe	343	.	A	B	.	.	.	.	-0.40	.	.	.	-0.60	0.27
Asp	344	.	.	.	.	T	T	.	-0.67	*	*	.	0.20	0.20
Ile	345	.	.	.	.	T	T	.	-0.93	*	*	.	0.20	0.30
Gly	346	.	.	.	.	T	T	.	-0.93	*	*	F	0.65	0.26
Gly	347	.	.	.	.	.	T	C	-0.82	*	*	F	1.05	0.21
Ser	348	.	.	.	.	.	.	C	-0.47	*	*	F	-0.05	0.30
Val	349	.	.	B	.	.	.	.	-1.17	*	*	F	0.05	0.30
Ser	350	.	.	B	.	.	T	.	-0.58	*	*	.	-0.20	0.26
Ala	351	.	.	B	.	.	T	.	-0.23	.	.	.	-0.08	0.26
Gly	352	.	.	B	.	.	T	.	-0.19	.	.	.	0.04	0.57
Phe	353	.	.	B	.	.	T	.	0.11	.	.	F	0.61	0.57
Ser	354	.	.	.	.	.	.	C	0.67	.	.	F	0.73	0.91
Asn	355	.	.	.	.	.	T	C	0.67	.	.	F	1.20	1.24
Ser	356	.	.	.	.	.	T	C	0.94	.	.	F	1.08	1.91
Asn	357	.	.	.	.	.	T	C	0.43	.	.	F	1.56	2.06
Ser	358	.	.	.	.	.	T	C	0.54	.	.	F	0.69	0.95
Ser	359	.	.	.	B	.	.	C	-0.04	.	.	F	0.17	0.72
Thr	360	.	.	B	B	.	.	.	-0.04	.	.	F	-0.45	0.31

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Val	361	.	.	B	B	.	.	.	0.22	.	.	.	-0.30	0.39
Ala	362	.	.	B	B	.	.	.	-0.08	.	*	.	-0.30	0.40
Ile	363	.	.	B	B	.	.	.	-0.59	.	*	.	-0.30	0.37
Asp	364	.	.	B	B	.	.	.	-0.59	.	*	.	-0.30	0.41
His	365	.	.	B	.	.	.	.	-1.09	.	*	.	-0.10	0.54
Ser	366	A	A	.	.	.	.	.	-0.82	.	*	.	-0.30	0.64
Leu	367	.	A	B	.	.	.	.	-0.58	*	*	.	-0.30	0.38
Ser	368	.	A	.	.	.	.	C	0.31	*	*	.	-0.40	0.28
Leu	369	.	A	.	.	.	.	C	0.42	.	.	.	-0.10	0.36
Ala	370	A	A	.	.	.	.	.	0.14	*	.	.	0.30	0.86
Gly	371	A	A	.	.	.	.	.	0.16	*	.	F	0.75	0.93
Glu	372	A	A	.	.	.	.	.	0.38	*	.	F	0.00	1.18
Arg	373	A	A	.	.	.	.	.	0.68	.	.	F	0.60	1.18
Thr	374	A	A	.	.	.	.	.	1.18	*	.	F	0.90	2.06
Trp	375	A	A	.	.	.	.	.	1.17	*	.	.	0.75	1.72
Ala	376	A	A	.	.	.	.	.	1.17	*	.	.	0.30	0.87
Glu	377	A	A	.	.	.	.	.	0.36	.	.	.	-0.60	0.60
Thr	378	A	A	.	.	.	.	.	0.24	.	.	.	-0.60	0.47
Met	379	A	A	.	.	.	.	.	0.24	.	.	.	-0.30	0.74
Gly	380	.	A	.	.	.	.	C	-0.06	.	.	.	-0.10	0.62
Leu	381	.	A	.	.	.	.	C	0.53	.	.	.	-0.40	0.43
Asn	382	A	A	.	.	.	.	.	0.22	.	.	F	-0.15	0.73
Thr	383	A	A	.	.	.	.	.	-0.06	*	.	F	0.60	1.07
Ala	384	A	A	.	.	.	.	.	0.66	.	*	F	0.00	1.31
Asp	385	A	A	.	.	.	.	.	0.19	.	*	F	0.90	1.59
Thr	386	A	A	.	.	.	.	.	1.00	.	*	F	0.45	0.91
Ala	387	A	A	.	.	.	.	.	0.41	*	*	F	0.60	1.45
Arg	388	A	A	.	.	.	.	.	0.72	*	*	.	0.30	0.88
Leu	389	A	A	.	.	.	.	.	0.42	.	*	.	0.30	0.98
Asn	390	A	.	.	.	.	T	.	0.53	*	*	.	0.10	0.68
Ala	391	.	.	B	.	.	T	.	0.60	*	*	.	0.70	0.68
Asn	392	.	.	B	.	.	T	.	0.33	*	*	.	-0.05	1.29
Ile	393	.	.	B	.	.	T	.	0.22	*	*	.	0.10	0.59
Arg	394	.	.	B	B	.	.	.	0.72	*	*	.	-0.30	0.95
Tyr	395	.	.	B	B	.	.	.	0.38	*	*	.	-0.30	0.85
Val	396	.	.	B	B	.	.	.	0.66	*	*	.	-0.15	1.20
Asn	397	.	.	B	.	.	T	.	0.07	*	*	F	0.25	0.88
Thr	398	.	.	B	.	.	T	.	0.74	*	*	F	-0.05	0.57
Gly	399	.	.	.	.	T	T	.	-0.26	*	.	F	0.80	1.19
Thr	400	.	.	B	.	.	T	.	-0.26	*	.	F	-0.05	0.52
Ala	401	.	.	B	B	.	.	.	0.60	*	.	F	-0.45	0.56
Pro	402	.	.	B	B	.	.	.	-0.26	*	.	.	-0.60	0.91
Ile	403	.	.	B	B	.	.	.	-0.76	*	.	.	-0.60	0.47
Tyr	404	.	.	B	B	.	.	.	-0.62	*	.	.	-0.60	0.38
Asn	405	.	.	B	B	.	.	.	-0.62	.	.	.	-0.60	0.38
Val	406	.	.	B	B	.	.	.	-0.34	.	.	.	-0.60	0.79
Leu	407	.	.	B	B	.	.	.	-0.43	.	.	.	-0.60	0.73
Pro	408	.	.	B	.	.	T	.	-0.36	.	.	F	-0.05	0.61
Thr	409	.	.	B	.	.	T	.	-0.97	.	.	F	-0.05	0.67
Thr	410	.	.	B	.	.	T	.	-1.78	.	.	F	-0.05	0.61
Ser	411	.	.	B	.	.	T	.	-1.27	.	.	F	-0.05	0.32
Leu	412	.	.	B	B	.	.	.	-0.41	.	.	.	-0.60	0.22
Val	413	.	.	B	B	.	.	.	-0.20	.	.	.	-0.30	0.31
Leu	414	.	.	B	B	.	.	.	0.11	.	.	.	-0.30	0.37
Gly	415	.	.	.	.	T	T	.	0.11	.	.	F	0.65	0.77
Lys	416	A	.	.	.	.	T	.	-0.40	.	.	F	0.40	1.50
Asn	417	A	.	.	.	.	T	.	-0.18	.	.	F	0.40	1.50
Gln	418	A	.	.	.	.	T	.	0.37	.	.	F	1.00	1.54
Thr	419	A	.	.	B	.	.	.	0.29	.	*	F	0.60	1.11
Leu	420	A	.	.	B	.	.	.	0.68	.	*	F	-0.45	0.48

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ala	421	A	.	.	B	.	.	.	0.04	.	*	.	-0.30	0.56
Thr	422	A	.	.	B	.	.	.	0.09	.	*	.	-0.30	0.39
Ile	423	A	.	.	B	.	.	.	0.09	.	*	.	0.30	0.95
Lys	424	A	.	.	B	.	.	.	0.40	.	*	F	0.90	1.62
Ala	425	A	A	.	.	.	.	.	1.21	.	*	F	0.90	1.81
Lys	426	A	A	.	.	.	.	.	0.99	.	*	F	0.90	4.47
Glu	427	A	A	.	.	.	.	.	1.00	*	*	F	0.90	1.84
Asn	428	A	A	.	.	.	.	.	1.89	*	*	F	0.90	2.44
Gln	429	A	A	.	B	.	.	.	0.96	*	.	F	0.90	2.12
Leu	430	A	A	.	B	.	.	.	0.73	.	.	F	0.45	0.86
Ser	431	.	A	B	B	.	.	.	0.10	.	.	F	-0.45	0.44
Gln	432	.	A	B	B	.	.	.	-0.11	.	.	.	-0.60	0.26
Ile	433	.	A	B	B	.	.	.	-0.11	*	.	.	-0.60	0.48
Leu	434	.	A	B	B	.	.	.	-0.11	.	.	.	-0.60	0.58
Ala	435	.	.	B	.	.	T	.	0.46	.	.	.	-0.20	0.54
Pro	436	.	.	B	.	.	T	.	0.51	.	.	F	0.10	1.20
Asn	437	.	.	.	.	T	T	.	0.30	.	.	F	0.50	2.27
Asn	438	.	.	.	.	T	T	.	0.89	.	.	F	0.50	3.48
Tyr	439	.	.	.	.	T	.	.	1.74	.	.	F	0.30	3.02
Tyr	440	.	.	B	.	.	T	.	2.33	.	.	F	0.40	3.75
Pro	441	.	.	.	.	T	T	.	1.73	.	.	F	0.80	3.75
Ser	442	.	.	.	.	T	T	.	1.14	.	.	F	0.50	1.97
Lys	443	.	.	B	.	.	T	.	0.93	.	.	F	0.40	1.27
Asn	444	.	A	B	.	.	.	.	0.29	*	.	F	0.60	1.27
Leu	445	.	A	B	.	.	.	.	-0.06	.	.	.	-0.30	0.67
Ala	446	.	A	B	.	.	.	.	-0.66	*	.	.	-0.30	0.34
Pro	447	.	A	B	.	.	.	.	-0.36	*	.	.	-0.60	0.17
Ile	448	.	A	B	.	.	.	.	-0.99	*	*	.	-0.60	0.34
Ala	449	A	A	.	.	.	.	.	-0.99	*	.	.	-0.60	0.34
Leu	450	.	A	B	.	.	.	.	-0.18	*	*	.	-0.60	0.38
Asn	451	.	A	B	.	.	.	.	0.41	*	*	.	0.04	0.90
Ala	452	A	A	.	.	.	.	.	-0.08	*	*	F	1.58	1.48
Gln	453	A	A	.	.	.	.	.	0.51	*	*	F	1.62	1.56
Asp	454	.	.	.	.	T	T	.	0.80	*	*	F	3.06	1.30
Asp	455	.	.	.	.	T	T	.	1.30	.	*	F	3.40	1.72
Phe	456	.	.	.	.	T	T	.	1.09	.	*	F	3.06	1.43
Ser	457	.	.	B	.	.	T	.	0.79	.	.	F	2.32	1.33
Ser	458	.	.	B	B	.	.	.	0.48	.	.	F	0.53	0.56
Thr	459	.	.	.	B	.	.	C	-0.12	.	.	F	0.09	0.93
Pro	460	.	.	.	B	.	.	C	-0.12	.	.	F	-0.25	0.69
Ile	461	.	.	.	B	T	.	.	0.33	.	*	F	-0.05	0.82
Thr	462	.	.	B	B	.	.	.	0.63	.	.	.	-0.60	0.89
Met	463	.	.	B	B	.	.	.	0.93	.	.	.	-0.60	0.93
Asn	464	.	.	B	.	.	T	.	0.54	*	.	.	-0.05	2.30
Tyr	465	.	.	B	.	.	T	.	-0.06	*	.	.	-0.05	1.38
Asn	466	.	.	.	.	.	T	C	0.83	*	*	.	0.15	1.15
Gln	467	A	.	.	.	.	T	.	0.33	.	*	.	-0.05	1.24
Phe	468	A	A	.	.	.	.	.	0.93	.	.	.	-0.60	0.65
Leu	469	A	A	.	.	.	.	.	0.98	*	.	.	0.30	0.70
Glu	470	A	A	.	.	.	.	.	0.91	.	.	.	0.30	0.81
Leu	471	A	A	.	.	.	.	.	0.96	.	.	.	0.45	1.35
Glu	472	A	A	.	.	.	.	.	0.96	.	.	F	0.90	3.27
Lys	473	A	A	.	.	.	.	.	0.84	.	*	F	0.90	3.27
Thr	474	A	A	.	.	.	.	.	1.77	.	*	F	0.90	3.27
Lys	475	A	A	.	.	.	.	.	0.96	.	*	F	0.90	3.70
Gln	476	A	A	.	.	.	.	.	1.77	.	*	F	0.90	1.53
Leu	477	A	A	.	.	.	.	.	1.46	.	*	.	0.98	1.77
Arg	478	.	A	B	.	.	.	.	1.41	.	*	.	1.21	1.27
Leu	479	.	A	B	.	.	.	.	1.72	*	*	.	1.44	1.23
Asp	480	.	.	B	.	.	T	.	0.82	*	*	F	2.22	2.58

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Thr	481	.	.	B	.	.	T	.	0.58	.	*	F	2.30	0.98
Asp	482	.	.	B	.	.	T	.	1.04	.	*	F	1.92	1.86
Gln	483	.	.	B	.	.	T	.	0.93	.	*	F	1.69	1.10
Val	484	.	.	B	B	.	.	.	0.86	.	.	.	0.31	1.23
Tyr	485	.	.	B	B	.	.	.	0.27	*	.	.	-0.07	0.52
Gly	486	.	.	B	B	.	.	.	0.27	*	.	.	-0.60	0.30
Asn	487	.	.	B	B	.	.	.	0.02	.	.	.	-0.60	0.58
Ile	488	.	.	B	B	.	.	.	0.02	.	*	.	-0.60	0.58
Ala	489	.	.	B	B	.	.	.	0.18	.	*	.	-0.60	0.95
Thr	490	.	.	B	B	.	.	.	0.42	.	.	.	-0.60	0.51
Tyr	491	.	.	B	.	.	.	.	0.77	*	.	.	-0.25	1.26
Asn	492	.	.	B	.	.	.	.	0.42	*	.	.	0.39	2.01
Phe	493	.	.	B	.	.	T	.	1.42	.	.	.	0.93	1.38
Glu	494	.	.	.	.	T	T	.	1.16	.	*	F	2.42	1.72
Asn	495	.	.	.	.	T	T	.	1.58	.	*	F	2.61	0.80
Gly	496	.	.	.	.	T	T	.	0.97	.	*	F	3.40	1.80
Arg	497	.	.	B	B	.	.	.	0.97	.	*	F	2.11	0.77
Val	498	.	.	B	B	.	.	.	1.36	.	*	F	1.77	0.80
Arg	499	.	.	B	B	.	.	.	1.01	.	*	.	1.55	1.17
Val	500	.	.	B	B	.	.	.	0.71	.	*	F	1.33	0.59
Asp	501	.	.	B	B	.	.	.	1.06	.	*	F	0.96	1.07
Thr	502	.	.	.	.	.	.	C	0.66	.	*	F	1.63	0.87
Gly	503	.	.	.	.	.	T	C	1.21	.	*	F	1.20	1.24
Ser	504	.	.	.	.	.	T	C	1.10	.	*	F	0.93	0.99
Asn	505	.	.	.	.	.	T	C	1.10	*	.	F	0.96	1.19
Trp	506	.	.	.	.	.	T	C	0.29	*	.	F	0.69	0.89
Ser	507	.	.	.	.	.	.	C	0.39	*	.	F	0.07	0.55
Glu	508	.	.	B	.	.	.	.	0.73	*	.	.	-0.40	0.53
Val	509	.	.	B	B	.	.	.	0.14	*	*	.	-0.60	0.87
Leu	510	.	.	B	B	.	.	.	0.14	*	.	F	-0.15	0.46
Pro	511	.	.	.	B	.	.	C	0.43	*	.	F	0.05	0.46
Gln	512	.	.	.	B	.	.	C	0.42	*	.	F	0.20	1.06
Ile	513	A	.	.	B	.	.	.	0.11	*	.	F	0.00	1.86
Gln	514	A	.	.	B	.	.	.	0.38	*	*	F	0.60	1.74
Glu	515	A	.	.	B	.	.	.	1.30	*	*	F	0.60	1.01
Thr	516	.	.	B	B	.	.	.	0.62	*	.	F	0.60	2.83
Thr	517	.	.	B	B	.	.	.	-0.27	*	*	F	0.60	1.15
Ala	518	.	.	B	B	.	.	.	-0.08	*	*	.	0.30	0.46
Arg	519	.	.	B	B	.	.	.	-0.08	*	*	.	-0.60	0.28
Ile	520	.	.	B	B	.	.	.	-0.42	*	*	.	-0.60	0.31
Ile	521	.	.	B	B	.	.	.	-0.07	*	*	.	-0.36	0.30
Phe	522	.	.	B	B	.	.	.	0.24	*	*	.	0.78	0.31
Asn	523	.	.	.	B	T	.	.	0.02	*	*	F	1.57	0.74
Gly	524	.	.	.	.	T	T	.	-0.09	*	*	F	2.21	0.87
Lys	525	.	.	.	.	.	T	C	-0.01	.	.	F	2.40	1.62
Asp	526	.	.	.	.	.	T	C	0.02	.	.	F	2.01	0.83
Leu	527	.	.	.	.	.	T	C	0.72	*	*	F	1.77	0.62
Asn	528	A	A	.	.	.	.	.	0.83	*	.	.	1.08	0.54
Leu	529	A	A	.	.	.	.	.	1.29	*	.	.	0.84	0.63
Val	530	A	A	.	.	.	.	.	0.36	*	.	.	0.75	1.50
Glu	531	A	A	.	.	.	.	.	-0.23	*	*	.	0.60	0.65
Arg	532	A	A	.	.	.	.	.	-0.01	*	.	.	0.30	0.80
Arg	533	A	A	.	.	.	.	.	-0.87	*	.	.	0.75	1.09
Ile	534	.	A	B	.	.	.	.	-0.06	*	.	.	0.60	0.47
Ala	535	.	A	B	.	.	.	.	0.59	*	.	.	0.30	0.38
Ala	536	.	A	B	.	.	.	.	0.29	.	.	.	-0.30	0.30
Val	537	.	A	B	.	.	.	.	0.18	*	.	.	-0.30	0.58
Asn	538	.	.	.	.	.	T	C	-0.14	.	.	F	1.65	0.96
Pro	539	.	.	.	.	.	T	C	-0.07	.	.	F	2.10	1.46
Ser	540	.	.	.	.	.	T	C	0.52	*	.	F	2.40	1.63

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Asp	541	.	.	.	.	.	T	C	0.80	.	.	F	3.00	1.75
Pro	542	.	.	.	.	.	.	C	1.34	.	.	F	2.50	1.64
Leu	543	.	.	B	.	.	.	.	1.39	.	.	F	2.00	1.76
Glu	544	.	.	B	.	.	.	.	1.39	.	.	F	1.70	2.11
Thr	545	A	.	.	.	.	.	.	1.69	.	.	F	1.10	2.11
Thr	546	A	.	.	.	.	.	.	1.09	.	.	F	1.10	4.27
Lys	547	A	.	.	.	.	T	.	0.99	.	*	F	1.30	2.44
Pro	548	A	.	.	.	.	T	.	0.99	.	*	F	1.00	2.44
Asp	549	A	.	.	.	.	T	.	1.03	.	*	F	1.00	1.39
Met	550	A	.	.	.	.	T	.	1.34	*	*	F	1.30	1.39
Thr	551	A	A	.	.	.	.	.	1.07	*	*	.	0.75	1.56
Leu	552	A	A	.	.	.	.	.	0.21	*	*	.	0.60	0.94
Lys	553	A	A	.	.	.	.	.	0.47	*	*	F	0.45	0.79
Glu	554	A	A	.	.	.	.	.	-0.42	*	*	F	0.90	1.09
Ala	555	A	A	.	.	.	.	.	-0.41	*	*	.	0.30	0.93
Leu	556	A	A	.	.	.	.	.	-0.80	*	*	.	0.60	0.47
Lys	557	A	A	.	.	.	.	.	-0.33	*	*	.	-0.30	0.23
Ile	558	A	A	.	.	.	.	.	-1.08	*	*	.	-0.60	0.23
Ala	559	A	A	.	.	.	.	.	-1.08	*	*	.	-0.60	0.24
Phe	560	.	A	B	.	.	.	.	-0.49	*	*	.	-0.60	0.19
Gly	561	.	A	B	.	.	.	.	0.11	.	*	.	-0.32	0.48
Phe	562	.	.	B	.	.	.	.	0.07	.	*	.	0.46	0.73
Asn	563	.	.	.	.	.	.	C	0.61	.	.	F	1.24	1.36
Glu	564	.	.	.	.	.	T	C	1.20	.	.	F	2.32	1.36
Pro	565	.	.	.	.	T	T	.	1.09	.	*	F	2.80	2.53
Asn	566	.	.	.	.	T	T	.	1.43	.	*	F	2.52	1.30
Gly	567	.	.	.	.	T	T	.	1.89	.	*	F	2.24	1.30
Asn	568	.	.	.	.	.	.	C	1.89	.	*	F	0.66	1.31
Leu	569	.	.	B	.	.	.	.	1.54	.	*	.	0.23	1.41
Gln	570	.	.	B	.	.	.	.	1.80	.	*	.	0.15	1.41
Tyr	571	.	.	B	.	.	T	.	1.80	*	*	.	0.85	1.76
Gln	572	.	.	B	.	.	T	.	1.26	*	*	F	1.80	3.56
Gly	573	.	.	B	.	.	T	.	0.94	*	*	F	2.00	1.44
Lys	574	.	.	B	.	.	T	.	1.76	*	*	F	1.80	1.33
Asp	575	.	A	B	.	.	.	.	1.06	*	*	F	1.50	1.33
Ile	576	.	A	B	.	.	.	.	1.30	.	*	F	1.30	1.16
Thr	577	.	A	B	.	.	.	.	0.60	*	*	F	0.95	0.97
Glu	578	.	A	B	.	.	.	.	0.94	*	*	.	0.30	0.50
Phe	579	.	A	B	.	.	.	.	0.20	*	*	.	-0.15	1.15
Asp	580	.	A	.	.	T	.	.	0.20	*	*	.	0.10	0.69
Phe	581	.	A	.	.	T	.	.	1.09	*	*	.	0.70	0.67
Asn	582	.	.	.	.	T	T	.	1.40	.	*	.	0.65	1.34
Phe	583	.	.	.	.	T	T	.	1.09	.	*	F	1.40	1.38
Asp	584	A	.	.	.	.	T	.	1.49	.	*	F	0.40	2.31
Gln	585	A	.	.	.	.	T	.	1.49	.	*	F	1.28	1.92
Gln	586	.	.	.	.	T	.	.	2.19	*	*	F	1.76	3.85
Thr	587	.	.	.	.	.	.	C	1.30	*	*	F	2.14	3.70
Ser	588	.	.	.	.	.	T	C	2.04	*	*	F	1.72	1.50
Gln	589	.	.	.	.	T	T	.	2.04	*	*	F	2.80	1.73
Asn	590	.	.	.	.	T	T	.	2.04	*	*	F	2.52	1.93
Ile	591	.	.	.	.	.	T	C	1.23	*	*	F	2.04	2.49
Lys	592	.	A	.	.	.	.	C	0.96	*	.	F	1.36	1.19
Asn	593	.	A	B	.	.	.	.	1.26	*	.	F	0.13	0.75
Gln	594	.	A	B	.	.	.	.	0.44	*	.	F	0.60	1.84
Leu	595	.	A	B	.	.	.	.	0.44	*	*	.	0.30	0.76
Ala	596	.	A	B	.	.	.	.	0.74	*	*	.	-0.30	0.76
Glu	597	A	A	.	.	.	.	.	0.39	.	.	.	-0.30	0.44
Leu	598	A	A	.	.	.	.	.	0.39	.	.	.	-0.30	0.78
Asn	599	A	A	.	.	.	.	.	-0.50	.	.	.	0.45	1.23
Ala	600	A	.	.	B	.	.	.	0.07	.	.	.	-0.30	0.50

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Thr	601	A	.	.	B	.	.	.	0.34	.	.	.	-0.60	0.95
Asn	602	A	.	.	B	.	.	.	-0.51	.	.	.	-0.60	0.85
Ile	603	.	.	B	B	.	.	.	-0.51	.	.	.	-0.60	0.63
Tyr	604	.	.	B	B	.	.	.	-0.51	*	.	.	-0.60	0.36
Thr	605	.	A	B	B	.	.	.	0.12	*	.	.	-0.60	0.37
Val	606	.	A	B	B	.	.	.	-0.46	*	*	.	-0.15	1.06
Leu	607	A	A	.	B	.	.	.	-0.41	*	*	.	-0.30	0.47
Asp	608	A	A	.	B	.	.	.	-0.33	*	*	F	0.45	0.66
Lys	609	A	A	.	B	.	.	.	-0.09	*	*	F	0.45	0.73
Ile	610	A	A	.	B	.	.	.	-0.37	*	*	F	0.90	1.42
Lys	611	A	A	.	B	.	.	.	0.53	*	*	F	0.75	0.86
Leu	612	A	A	.	.	.	.	.	0.74	.	*	F	0.75	0.86
Asn	613	A	A	.	.	.	.	.	0.74	.	*	.	0.45	1.22
Ala	614	A	A	.	.	.	.	.	-0.19	.	*	.	0.30	0.98
Lys	615	A	A	.	.	.	.	.	-0.11	.	*	.	-0.30	0.83
Met	616	A	.	.	B	.	.	.	-1.04	*	*	.	-0.30	0.43
Asn	617	A	.	.	B	.	.	.	-0.12	*	*	.	-0.60	0.30
Ile	618	A	.	.	B	.	.	.	-0.12	.	*	.	-0.30	0.29
Leu	619	A	.	.	B	.	.	.	0.51	.	*	.	-0.30	0.49
Ile	620	A	.	.	B	.	.	.	0.58	.	*	.	0.60	0.61
Arg	621	A	.	.	B	.	.	.	0.48	*	*	.	0.75	1.70
Asp	622	A	.	.	B	.	.	.	0.44	*	*	F	0.90	1.78
Lys	623	.	A	B	.	.	.	.	1.09	*	*	F	0.90	3.46
Arg	624	.	A	B	.	.	.	.	1.90	*	.	.	1.03	2.77
Phe	625	.	A	B	.	.	.	.	2.90	.	.	.	1.31	2.77
His	626	.	A	.	.	T	.	.	2.79	.	.	.	1.99	2.71
Tyr	627	.	A	.	.	T	.	.	2.79	.	*	.	2.27	2.23
Asp	628	.	.	.	.	T	T	.	1.86	*	.	F	2.80	4.13
Arg	629	.	.	.	.	T	T	.	1.16	.	*	F	2.52	2.13
Asn	630	.	.	.	.	T	T	.	1.00	.	.	F	2.24	1.37
Asn	631	.	.	.	.	T	T	.	0.69	.	.	.	1.66	0.61
Ile	632	.	.	B	B	.	.	.	0.34	.	*	.	-0.02	0.31
Ala	633	.	.	B	B	.	.	.	0.34	.	*	.	-0.60	0.19
Val	634	.	.	B	B	.	.	.	0.23	.	*	.	-0.30	0.20
Gly	635	A	.	.	B	.	.	.	-0.07	*	.	.	0.30	0.50
Ala	636	A	A	.	.	.	.	.	-0.92	.	*	F	0.75	0.66
Asp	637	A	A	.	.	.	.	.	-0.89	*	.	F	0.45	0.66
Glu	638	A	A	.	B	.	.	.	-0.26	*	.	F	0.45	0.49
Ser	639	A	A	.	B	.	.	.	0.60	*	.	F	0.75	0.98
Val	640	A	A	.	B	.	.	.	0.36	*	.	F	0.90	1.02
Val	641	A	A	.	B	.	.	.	0.91	*	.	.	0.60	0.59
Lys	642	A	A	.	B	.	.	.	1.02	*	.	.	0.30	0.60
Glu	643	A	A	.	.	.	.	.	1.02	*	.	.	0.75	1.59
Ala	644	A	A	.	.	.	.	.	0.47	*	.	.	0.75	3.70
His	645	A	A	.	.	.	.	.	0.43	*	.	.	0.75	1.37
Arg	646	A	A	.	B	.	.	.	1.29	*	.	.	0.60	0.56
Glu	647	A	A	.	B	.	.	.	0.94	*	.	.	0.54	0.89
Val	648	A	A	.	B	.	.	.	0.64	*	.	.	0.78	0.87
Ile	649	A	A	.	B	.	.	.	0.92	*	.	.	1.02	0.60
Asn	650	.	.	.	.	.	T	C	0.96	*	.	F	2.01	0.50
Ser	651	.	.	.	.	.	T	C	0.50	*	.	F	2.40	1.16
Ser	652	.	.	.	.	.	T	C	-0.31	*	.	F	2.16	1.64
Thr	653	.	.	.	.	.	T	C	-0.27	.	.	F	1.77	0.84
Glu	654	A	A	.	.	.	.	.	-0.19	.	.	F	0.33	0.52
Gly	655	A	A	.	.	.	.	.	-0.19	.	.	F	0.09	0.32
Leu	656	A	A	.	.	.	.	.	-0.78	.	*	.	-0.30	0.35
Leu	657	A	A	.	.	.	.	.	-0.48	*	.	.	-0.60	0.14
Leu	658	A	A	.	.	.	.	.	-0.12	*	.	.	-0.60	0.24
Asn	659	A	A	.	.	.	.	.	-0.12	*	*	.	-0.30	0.59
Ile	660	A	A	.	.	.	.	.	-0.67	*	*	F	0.90	1.19

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Asp	661	A	.	.	.	.	T	.	0.26	*	*	F	1.30	1.01
Lys	662	A	.	.	.	.	T	.	1.11	*	*	F	1.30	1.23
Asp	663	A	.	.	.	.	T	.	1.03	*	*	F	1.30	3.51
Ile	664	A	.	.	.	.	T	.	0.22	*	*	F	1.30	1.47
Arg	665	.	.	B	B	.	.	.	0.81	*	*	F	0.75	0.61
Lys	666	.	.	B	B	.	.	.	0.47	*	.	F	0.75	0.49
Ile	667	.	.	B	B	.	.	.	0.18	*	.	.	0.30	0.69
Leu	668	.	.	B	.	.	T	.	-0.71	*	*	.	0.70	0.55
Ser	669	.	.	B	.	.	T	.	-0.68	*	.	.	-0.20	0.19
Gly	670	.	.	B	.	.	T	.	-0.79	*	.	.	-0.20	0.20
Tyr	671	.	.	B	.	.	T	.	-1.72	*	.	.	-0.20	0.43
Ile	672	.	.	B	B	.	.	.	-0.83	*	.	.	-0.60	0.22
Val	673	.	.	B	B	.	.	.	-0.02	*	.	.	-0.30	0.39
Glu	674	.	.	B	B	.	.	.	-0.03	.	.	.	0.30	0.42
Ile	675	.	.	B	B	.	.	.	0.31	.	.	.	0.30	0.86
Glu	676	A	.	.	B	.	.	.	0.21	.	.	F	0.90	2.01
Asp	677	A	.	.	.	.	T	.	0.29	.	.	F	1.30	1.15
Thr	678	A	.	.	.	.	T	.	1.19	.	*	F	1.30	1.35
Glu	679	A	.	.	.	.	T	.	1.19	.	.	F	1.30	1.56
Gly	680	A	.	.	.	.	T	.	1.22	*	.	F	1.30	1.62
Leu	681	A	A	.	.	.	.	.	0.33	*	.	F	0.75	0.83
Lys	682	A	A	.	.	.	.	.	0.33	*	.	F	0.75	0.34
Glu	683	A	A	.	.	.	.	.	0.64	*	*	.	0.30	0.55
Val	684	A	A	.	.	.	.	.	0.76	*	*	.	0.75	1.11
Ile	685	A	A	.	.	.	.	.	0.86	*	*	.	0.75	1.09
Asn	686	A	.	.	.	.	T	.	1.67	*	*	.	1.00	0.98
Asp	687	A	.	.	.	.	T	.	1.02	*	*	.	1.15	2.21
Arg	688	A	.	.	.	.	T	.	0.21	*	.	.	1.15	3.12
Tyr	689	A	.	.	.	.	T	.	1.07	.	.	.	1.15	1.60
Asp	690	.	.	B	.	.	.	.	1.07	.	*	.	0.95	1.54
Met	691	.	.	B	B	.	.	.	0.77	.	.	.	-0.30	0.55
Leu	692	.	.	B	B	.	.	.	0.47	.	.	.	-0.60	0.47
Asn	693	.	.	B	B	.	.	.	-0.46	.	.	.	-0.30	0.38
Ile	694	.	.	B	B	.	.	.	-0.10	.	.	.	-0.60	0.32
Ser	695	.	.	B	B	.	.	.	-0.10	.	.	F	0.19	0.75
Ser	696	.	.	B	B	.	.	.	0.50	.	.	F	1.13	0.81
Leu	697	.	.	B	B	.	.	.	0.97	*	*	F	1.62	1.92
Arg	698	A	.	.	.	.	T	.	1.01	*	*	F	2.66	1.42
Gln	699	.	.	.	.	T	T	.	1.59	*	*	F	3.40	2.12
Asp	700	.	.	.	.	T	T	.	1.19	*	*	F	3.06	3.71
Gly	701	.	.	.	.	T	T	.	0.60	*	*	F	2.72	1.64
Lys	702	.	.	B	.	.	.	.	1.41	*	*	F	1.33	0.66
Thr	703	.	.	B	.	.	.	.	0.60	*	*	F	1.29	0.66
Phe	704	.	A	B	.	.	.	.	0.64	*	*	.	-0.30	0.58
Ile	705	.	A	B	.	.	.	.	0.69	*	*	.	0.30	0.58
Asp	706	A	A	.	.	.	.	.	0.79	*	*	.	0.64	0.80
Phe	707	A	A	.	.	.	.	.	0.74	*	*	.	0.53	1.46
Lys	708	A	A	.	.	.	.	.	1.06	*	.	F	1.62	3.34
Lys	709	.	A	.	.	T	.	.	1.80	*	.	F	2.66	3.34
Tyr	710	.	.	.	.	T	T	.	1.88	*	*	F	3.40	7.72
Asn	711	.	.	.	.	T	T	.	1.67	*	*	F	3.06	3.18
Asp	712	.	.	.	.	T	T	.	1.56	*	*	F	2.72	2.46
Lys	713	.	.	B	.	.	T	.	1.27	*	*	F	1.68	1.29
Leu	714	.	.	B	B	.	.	.	0.33	*	*	.	0.79	1.26
Pro	715	.	.	B	B	.	.	.	0.28	*	*	.	-0.30	0.53
Leu	716	.	.	B	B	.	.	.	0.28	*	*	.	-0.60	0.35
Tyr	717	.	.	B	B	.	.	.	0.07	*	.	.	-0.60	0.69
Ile	718	.	.	B	B	.	.	.	0.02	.	*	.	-0.60	0.69
Ser	719	.	.	B	B	.	.	.	0.59	.	*	.	-0.45	1.35
Asn	720	.	.	B	.	.	T	.	0.84	.	*	F	0.10	1.35

Table 2 (con't)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Pro	721	.	.	.	.	T	T	.	0.80	.	*	F	0.80	3.85
Asn	722	.	.	.	.	T	T	.	1.04	.	*	F	0.80	2.13
Tyr	723	.	.	.	.	T	T	.	1.08	.	*	F	0.80	2.13
Lys	724	.	.	B	B	.	.	.	1.13	.	*	.	-0.15	1.02
Val	725	.	.	B	B	.	.	.	0.54	.	*	.	-0.60	1.00
Asn	726	.	.	B	B	.	.	.	-0.10	.	*	.	-0.60	0.64
Val	727	.	.	B	B	.	.	.	-0.41	.	*	.	-0.60	0.24
Tyr	728	.	.	B	B	.	.	.	-0.12	.	*	.	-0.60	0.46
Ala	729	.	.	B	B	.	.	.	-0.17	.	*	.	-0.60	0.58
Val	730	A	.	.	B	.	.	.	0.69	.	*	.	-0.15	1.35
Thr	731	A	.	.	B	.	.	.	0.38	.	.	F	0.60	1.38
Lys	732	.	.	B	B	.	.	.	0.34	.	.	F	0.60	1.97
Glu	733	.	.	B	B	.	.	.	-0.30	*	.	F	0.60	1.86
Asn	734	.	.	B	B	.	.	.	0.29	*	.	F	0.45	0.91
Thr	735	.	.	B	B	.	.	.	0.93	.	.	F	0.45	0.73
Ile	736	.	.	B	B	.	.	.	0.94	.	.	.	-0.30	0.65
Ile	737	.	.	B	B	.	.	.	0.90	.	.	.	-0.26	0.54
Asn	738	.	.	B	.	.	T	.	0.90	*	.	F	0.93	0.65
Pro	739	.	.	.	.	.	T	C	0.56	*	.	F	2.22	1.49
Ser	740	.	.	.	.	.	T	C	0.87	*	.	F	2.56	2.11
Glu	741	.	.	.	.	T	T	.	1.44	*	.	F	3.40	2.19
Asn	742	.	.	.	.	T	T	.	2.03	.	*	F	3.06	2.04
Gly	743	.	.	.	.	T	T	.	1.72	.	.	F	2.72	2.04
Asp	744	.	.	.	.	T	T	.	1.93	.	.	F	2.38	1.70
Thr	745	.	.	.	.	.	T	C	1.89	*	.	F	1.54	1.70
Ser	746	.	.	.	.	.	T	C	1.00	*	.	F	1.20	1.70
Thr	747	A	.	.	.	.	T	.	1.04	*	.	F	0.85	0.71
Asn	748	A	.	.	.	.	T	.	1.43	*	.	F	0.85	0.99
Gly	749	A	.	.	.	.	T	.	0.54	*	.	F	1.30	1.48
Ile	750	A	.	.	B	.	.	.	0.04	*	.	F	0.45	0.72
Lys	751	.	.	B	B	.	.	.	-0.54	*	.	F	0.45	0.37
Lys	752	.	.	B	B	.	.	.	-0.93	*	.	F	-0.15	0.26
Ile	753	.	.	B	B	.	.	.	-1.23	*	.	.	-0.60	0.32
Leu	754	.	.	B	B	.	.	.	-0.84	*	.	.	-0.30	0.22
Ile	755	.	.	B	B	.	.	.	0.09	*	.	.	-0.30	0.22
Phe	756	.	.	B	B	.	.	.	-0.30	*	.	.	0.04	0.62
Ser	757	.	.	.	.	.	T	C	-0.59	*	.	F	1.73	0.74
Lys	758	.	.	.	.	T	T	.	0.30	*	.	F	1.82	1.65
Lys	759	.	.	.	.	.	T	C	0.22	*	.	F	2.86	3.30
Gly	760	.	.	.	.	T	T	.	0.77	*	.	F	3.40	1.73
Tyr	761	.	.	.	.	T	.	.	1.08	*	.	F	2.71	0.85
Glu	762	.	.	B	.	.	.	.	0.99	*	.	.	1.52	0.55
Ile	763	.	.	B	.	.	.	.	0.56	*	.	.	0.58	0.71
Gly	764	.	.	B	.	.	.	.	0.12	.	.	.	0.24	0.58

[0076] In another aspect, the invention provides an antibody that binds a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide described herein. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention.

[0077] As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science 219:*660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

[0078] Antigenic epitope-bearing peptides and polypeptides are therefore useful to raise antibodies, including monoclonal antibodies, that bind to a PA polypeptide of the invention. See, for instance, Wilson *et al., Cell 37:*767-778 (1984) at 777. Antigenic epitope-bearing peptides and polypeptides preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of SEQ ID NO:2.

[0079] Antibodies of the invention may bind one or more antigenic PA polypeptides or peptides including, but not limited to: a polypeptide comprising amino acid residues from about 39 to about 45 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 129 to about 134 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 151 to about 157 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 168 to about 172 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 189 to about 195 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 203 to about 213 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 225 to about 230 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 246 to about 253 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 259 to about 264 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 273 to about 280 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 302 to about

307 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 309 to about 314 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 319 to about 331 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 452 to about 457 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 480 to about 483 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 494 to about 498 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 523 to about 527 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 538 to about 544 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 564 to about 567 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 572 to about 575 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 587 to about 591 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 626 to about 631 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 650 to about 653 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 697 to about 701 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 708 to about 713 of SEQ ID NO:2; a polypeptide comprising amino acid residues from about 739 to about 745 of SEQ ID NO:2; and/or a polypeptide comprising amino acid residues from about 757 to about 762 of SEQ ID NO:2. In this context "about" includes the particularly recited range, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either terminus or at both termini. Epitope-bearing PA peptides and polypeptides may be produced by any conventional means. Houghten, R.A., "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids," *Proc. Natl. Acad. Sci. USA 82*:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

[0080] As one of skill in the art will appreciate, PA polypeptides and the epitope-bearing fragments thereof described herein can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al., Nature 331*:84- 86 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part

can also be more efficient in binding and neutralizing other molecules than the monomeric PA protein or protein fragment alone (Fountoulakis *et al., J Biochem 270*:3958-3964 (1995)). Thus, antibodies of the invention may bind the PA moiety of fusion proteins that comprise all or a portion of a PA polypeptide.

[0081] Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or "muteins" including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. Antibodies of the present invention may also bind such modified PA polypeptides or PA polypeptide fragments or variants.

[0082] For instance, for many proteins, it is known in the art that one or more amino acids may be deleted from the N-terminus or C-terminus without substantial loss of biological function, or loss of the ability to be bound by a specific antibody. For instance, Ron *et al., J. Biol. Chem., 268*:2984-2988 (1993) reported modified KGF proteins that had heparin binding activity even if 3, 8, or 27 amino-terminal amino acid residues were missing.

[0083] However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind EF or LF may still be retained. For example, the ability of shortened PA polypeptides to induce and/or bind to antibodies which recognize the complete or mature forms of the PA polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a PA polypeptide with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six PA amino acid residues may often evoke an immune response.

[0084] Accordingly, the present invention further provides antibodies that bind polypeptides having one or more residues deleted from the amino terminus of the PA amino acid sequence of SEQ ID NO:2 up to the serine residue at position number 463. In

particular, the present invention provides antibodies that bind polypeptides comprising the amino acid sequence of residues n^1-764 of SEQ ID NO:2, where n^1 is an integer from 31 to 759 corresponding to the position of the amino acid residue in SEQ ID NO:2.

[0085] More in particular, the invention provides antibodies that bind polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of V-31 to G-764; K-32 to G-764; Q-33 to G-764; E-34 to G-764; N-35 to G-764; R-36 to G-764; L-37 to G-764; L-38 to G-764; N-39 to G-764; E-40 to G-764; S-41 to G-764; E-42 to G-764; S-43 to G-764; S-44 to G-764; S-45 to G-764; Q-46 to G-764; G-47 to G-764; L-48 to G-764; L-49 to G-764; G-50 to G-764; Y-51 to G-764; Y-52 to G-764; F-53 to G-764; S-54 to G-764; D-55 to G-764; L-56 to G-764; N-57 to G-764; F-58 to G-764; Q-59 to G-764; A-60 to G-764; P-61 to G-764; M-62 to G-764; V-63 to G-764; V-64 to G-764; T-65 to G-764; S-66 to G-764; S-67 to G-764; T-68 to G-764; T-69 to G-764; G-70 to G-764; D-71 to G-764; L-72 to G-764; S-73 to G-764; I-74 to G-764; P-75 to G-764; S-76 to G-764; S-77 to G-764; E-78 to G-764; L-79 to G-764; E-80 to G-764; N-81 to G-764; I-82 to G-764; P-83 to G-764; S-84 to G-764; E-85 to G-764; N-86 to G-764; Q-87 to G-764; Y-88 to G-764; F-89 to G-764; Q-90 to G-764; S-91 to G-764; A-92 to G-764; I-93 to G-764; W-94 to G-764; S-95 to G-764; G-96 to G-764; F-97 to G-764; I-98 to G-764; K-99 to G-764; V-100 to G-764; K-101 to G-764; K-102 to G-764; S-103 to G-764; D-104 to G-764; E-105 to G-764; Y-106 to G-764; T-107 to G-764; F-108 to G-764; A-109 to G-764; T-110 to G-764; S-111 to G-764; A-112 to G-764; D-113 to G-764; N-114 to G-764; H-115 to G-764; V-116 to G-764; T-117 to G-764; M-118 to G-764; W-119 to G-764; V-120 to G-764; D-121 to G-764; D-122 to G-764; Q-123 to G-764; E-124 to G-764; V-125 to G-764; I-126 to G-764; N-127 to G-764; K-128 to G-764; A-129 to G-764; S-130 to G-764; N-131 to G-764; S-132 to G-764; N-133 to G-764; K-134 to G-764; I-135 to G-764; R-136 to G-764; L-137 to G-764; E-138 to G-764; K-139 to G-764; G-140 to G-764; R-141 to G-764; L-142 to G-764; Y-143 to G-764; Q-144 to G-764; I-145 to G-764; K-146 to G-764; I-147 to G-764; Q-148 to G-764; Y-149 to G-764; Q-150 to G-764; R-151 to G-764; E-152 to G-764; N-153 to G-764; P-154 to G-764; T-155 to G-764; E-156 to G-764; K-157 to G-764; G-158 to G-764; L-159 to G-764; D-160 to G-764; F-161 to G-764; K-162 to G-764; L-163 to G-764; Y-164 to G-764; W-165 to G-764; T-166 to G-764; D-167 to G-764; S-168 to G-764; Q-169 to G-764; N-170 to G-764; K-171 to G-764; K-172 to G-764; E-173 to G-764; V-174 to G-764; I-175 to G-764; S-176 to G-764; S-177 to G-764; D-178 to G-764; N-179 to G-764; L-180 to G-764; Q-181 to G-764; L-182 to G-764;

P-183 to G-764; E-184 to G-764; L-185 to G-764; K-186 to G-764; Q-187 to G-764; K-188 to G-764; S-189 to G-764; S-190 to G-764; N-191 to G-764; S-192 to G-764; R-193 to G-764; K-194 to G-764; K-195 to G-764; R-196 to G-764; S-197 to G-764; T-198 to G-764; S-199 to G-764; A-200 to G-764; G-201 to G-764; P-202 to G-764; T-203 to G-764; V-204 to G-764; P-205 to G-764; D-206 to G-764; R-207 to G-764; D-208 to G-764; N-209 to G-764; D-210 to G-764; G-211 to G-764; I-212 to G-764; P-213 to G-764; D-214 to G-764; S-215 to G-764; L-216 to G-764; E-217 to G-764; V-218 to G-764; E-219 to G-764; G-220 to G-764; Y-221 to G-764; T-222 to G-764; V-223 to G-764; D-224 to G-764; V-225 to G-764; K-226 to G-764; N-227 to G-764; K-228 to G-764; R-229 to G-764; T-230 to G-764; F-231 to G-764; L-232 to G-764; S-233 to G-764; P-234 to G-764; W-235 to G-764; I-236 to G-764; S-237 to G-764; N-238 to G-764; I-239 to G-764; H-240 to G-764; E-241 to G-764; K-242 to G-764; K-243 to G-764; G-244 to G-764; L-245 to G-764; T-246 to G-764; K-247 to G-764; Y-248 to G-764; K-249 to G-764; S-250 to G-764; S-251 to G-764; P-252 to G-764; E-253 to G-764; K-254 to G-764; W-255 to G-764; S-256 to G-764; T-257 to G-764; A-258 to G-764; S-259 to G-764; D-260 to G-764; P-261 to G-764; Y-262 to G-764; S-263 to G-764; D-264 to G-764; F-265 to G-764; E-266 to G-764; K-267 to G-764; V-268 to G-764; T-269 to G-764; G-270 to G-764; R-271 to G-764; I-272 to G-764; D-273 to G-764; K-274 to G-764; N-275 to G-764; V-276 to G-764; S-277 to G-764; P-278 to G-764; E-279 to G-764; A-280 to G-764; R-281 to G-764; H-282 to G-764; P-283 to G-764; L-284 to G-764; V-285 to G-764; A-286 to G-764; A-287 to G-764; Y-288 to G-764; P-289 to G-764; I-290 to G-764; V-291 to G-764; H-292 to G-764; V-293 to G-764; D-294 to G-764; M-295 to G-764; E-296 to G-764; N-297 to G-764; I-298 to G-764; I-299 to G-764; L-300 to G-764; S-301 to G-764; K-302 to G-764; N-303 to G-764; E-304 to G-764; D-305 to G-764; Q-306 to G-764; S-307 to G-764; T-308 to G-764; Q-309 to G-764; N-310 to G-764; T-311 to G-764; D-312 to G-764; S-313 to G-764; Q-314 to G-764; T-315 to G-764; R-316 to G-764; T-317 to G-764; I-318 to G-764; S-319 to G-764; K-320 to G-764; N-321 to G-764; T-322 to G-764; S-323 to G-764; T-324 to G-764; S-325 to G-764; R-326 to G-764; T-327 to G-764; H-328 to G-764; T-329 to G-764; S-330 to G-764; E-331 to G-764; V-332 to G-764; H-333 to G-764; G-334 to G-764; N-335 to G-764; A-336 to G-764; E-337 to G-764; V-338 to G-764; H-339 to G-764; A-340 to G-764; S-341 to G-764; F-342 to G-764; F-343 to G-764; D-344 to G-764; I-345 to G-764; G-346 to G-764; G-347 to G-764; S-348 to G-764; V-349 to G-764; S-350 to G-764; A-351 to G-764; G-352 to G-764; F-353 to G-764; S-354 to G-764; N-355 to G-764; S-

356 to G-764; N-357 to G-764; S-358 to G-764; S-359 to G-764; T-360 to G-764; V-361 to G-764; A-362 to G-764; I-363 to G-764; D-364 to G-764; H-365 to G-764; S-366 to G-764; L-367 to G-764; S-368 to G-764; L-369 to G-764; A-370 to G-764; G-371 to G-764; E-372 to G-764; R-373 to G-764; T-374 to G-764; W-375 to G-764; A-376 to G-764; E-377 to G-764; T-378 to G-764; M-379 to G-764; G-380 to G-764; L-381 to G-764; N-382 to G-764; T-383 to G-764; A-384 to G-764; D-385 to G-764; T-386 to G-764; A-387 to G-764; R-388 to G-764; L-389 to G-764; N-390 to G-764; A-391 to G-764; N-392 to G-764; I-393 to G-764; R-394 to G-764; Y-395 to G-764; V-396 to G-764; N-397 to G-764; T-398 to G-764; G-399 to G-764; T-400 to G-764; A-401 to G-764; P-402 to G-764; I-403 to G-764; Y-404 to G-764; N-405 to G-764; V-406 to G-764; L-407 to G-764; P-408 to G-764; T-409 to G-764; T-410 to G-764; S-411 to G-764; L-412 to G-764; V-413 to G-764; L-414 to G-764; G-415 to G-764; K-416 to G-764; N-417 to G-764; Q-418 to G-764; T-419 to G-764; L-420 to G-764; A-421 to G-764; T-422 to G-764; I-423 to G-764; K-424 to G-764; A-425 to G-764; K-426 to G-764; E-427 to G-764; N-428 to G-764; Q-429 to G-764; L-430 to G-764; S-431 to G-764; Q-432 to G-764; I-433 to G-764; L-434 to G-764; A-435 to G-764; P-436 to G-764; N-437 to G-764; N-438 to G-764; Y-439 to G-764; Y-440 to G-764; P-441 to G-764; S-442 to G-764; K-443 to G-764; N-444 to G-764; L-445 to G-764; A-446 to G-764; P-447 to G-764; I-448 to G-764; A-449 to G-764; L-450 to G-764; N-451 to G-764; A-452 to G-764; Q-453 to G-764; D-454 to G-764; D-455 to G-764; F-456 to G-764; S-457 to G-764; S-458 to G-764; T-459 to G-764; P-460 to G-764; I-461 to G-764; T-462 to G-764; M-463 to G-764; N-464 to G-764; Y-465 to G-764; N-466 to G-764; Q-467 to G-764; F-468 to G-764; L-469 to G-764; E-470 to G-764; L-471 to G-764; E-472 to G-764; K-473 to G-764; T-474 to G-764; K-475 to G-764; Q-476 to G-764; L-477 to G-764; R-478 to G-764; L-479 to G-764; D-480 to G-764; T-481 to G-764; D-482 to G-764; Q-483 to G-764; V-484 to G-764; Y-485 to G-764; G-486 to G-764; N-487 to G-764; I-488 to G-764; A-489 to G-764; T-490 to G-764; Y-491 to G-764; N-492 to G-764; F-493 to G-764; E-494 to G-764; N-495 to G-764; G-496 to G-764; R-497 to G-764; V-498 to G-764; R-499 to G-764; V-500 to G-764; D-501 to G-764; T-502 to G-764; G-503 to G-764; S-504 to G-764; N-505 to G-764; W-506 to G-764; S-507 to G-764; E-508 to G-764; V-509 to G-764; L-510 to G-764; P-511 to G-764; Q-512 to G-764; I-513 to G-764; Q-514 to G-764; E-515 to G-764; T-516 to G-764; T-517 to G-764; A-518 to G-764; R-519 to G-764; I-520 to G-764; I-521 to G-764; F-522 to G-764; N-523 to G-764; G-524 to G-764; K-525 to G-764; D-526 to G-764; L-527 to G-764; N-528 to G-

764; L-529 to G-764; V-530 to G-764; E-531 to G-764; R-532 to G-764; R-533 to G-764; I-534 to G-764; A-535 to G-764; A-536 to G-764; V-537 to G-764; N-538 to G-764; P-539 to G-764; S-540 to G-764; D-541 to G-764; P-542 to G-764; L-543 to G-764; E-544 to G-764; T-545 to G-764; T-546 to G-764; K-547 to G-764; P-548 to G-764; D-549 to G-764; M-550 to G-764; T-551 to G-764; L-552 to G-764; K-553 to G-764; E-554 to G-764; A-555 to G-764; L-556 to G-764; K-557 to G-764; I-558 to G-764; A-559 to G-764; F-560 to G-764; G-561 to G-764; F-562 to G-764; N-563 to G-764; E-564 to G-764; P-565 to G-764; N-566 to G-764; G-567 to G-764; N-568 to G-764; L-569 to G-764; Q-570 to G-764; Y-571 to G-764; Q-572 to G-764; G-573 to G-764; K-574 to G-764; D-575 to G-764; I-576 to G-764; T-577 to G-764; E-578 to G-764; F-579 to G-764; D-580 to G-764; F-581 to G-764; N-582 to G-764; F-583 to G-764; D-584 to G-764; Q-585 to G-764; Q-586 to G-764; T-587 to G-764; S-588 to G-764; Q-589 to G-764; N-590 to G-764; I-591 to G-764; K-592 to G-764; N-593 to G-764; Q-594 to G-764; L-595 to G-764; A-596 to G-764; E-597 to G-764; L-598 to G-764; N-599 to G-764; A-600 to G-764; T-601 to G-764; N-602 to G-764; I-603 to G-764; Y-604 to G-764; T-605 to G-764; V-606 to G-764; L-607 to G-764; D-608 to G-764; K-609 to G-764; I-610 to G-764; K-611 to G-764; L-612 to G-764; N-613 to G-764; A-614 to G-764; K-615 to G-764; M-616 to G-764; N-617 to G-764; I-618 to G-764; L-619 to G-764; I-620 to G-764; R-621 to G-764; D-622 to G-764; K-623 to G-764; R-624 to G-764; F-625 to G-764; H-626 to G-764; Y-627 to G-764; D-628 to G-764; R-629 to G-764; N-630 to G-764; N-631 to G-764; I-632 to G-764; A-633 to G-764; V-634 to G-764; G-635 to G-764; A-636 to G-764; D-637 to G-764; E-638 to G-764; S-639 to G-764; V-640 to G-764; V-641 to G-764; K-642 to G-764; E-643 to G-764; A-644 to G-764; H-645 to G-764; R-646 to G-764; E-647 to G-764; V-648 to G-764; I-649 to G-764; N-650 to G-764; S-651 to G-764; S-652 to G-764; T-653 to G-764; E-654 to G-764; G-655 to G-764; L-656 to G-764; L-657 to G-764; L-658 to G-764; N-659 to G-764; I-660 to G-764; D-661 to G-764; K-662 to G-764; D-663 to G-764; I-664 to G-764; R-665 to G-764; K-666 to G-764; I-667 to G-764; L-668 to G-764; S-669 to G-764; G-670 to G-764; Y-671 to G-764; I-672 to G-764; V-673 to G-764; E-674 to G-764; I-675 to G-764; E-676 to G-764; D-677 to G-764; T-678 to G-764; E-679 to G-764; G-680 to G-764; L-681 to G-764; K-682 to G-764; E-683 to G-764; V-684 to G-764; I-685 to G-764; N-686 to G-764; D-687 to G-764; R-688 to G-764; Y-689 to G-764; D-690 to G-764; M-691 to G-764; L-692 to G-764; N-693 to G-764; I-694 to G-764; S-695 to G-764; S-696 to G-764; L-697 to G-764; R-698 to G-764; Q-699 to G-764; D-700 to G-764;

G-701 to G-764; K-702 to G-764; T-703 to G-764; F-704 to G-764; I-705 to G-764; D-706 to G-764; F-707 to G-764; K-708 to G-764; K-709 to G-764; Y-710 to G-764; N-711 to G-764; D-712 to G-764; K-713 to G-764; L-714 to G-764; P-715 to G-764; L-716 to G-764; Y-717 to G-764; I-718 to G-764; S-719 to G-764; N-720 to G-764; P-721 to G-764; N-722 to G-764; Y-723 to G-764; K-724 to G-764; V-725 to G-764; N-726 to G-764; V-727 to G-764; Y-728 to G-764; A-729 to G-764; V-730 to G-764; T-731 to G-764; K-732 to G-764; E-733 to G-764; N-734 to G-764; T-735 to G-764; I-736 to G-764; I-737 to G-764; N-738 to G-764; P-739 to G-764; S-740 to G-764; E-741 to G-764; N-742 to G-764; G-743 to G-764; D-744 to G-764; T-745 to G-764; S-746 to G-764; T-747 to G-764; N-748 to G-764; G-749 to G-764; I-750 to G-764; K-751 to G-764; K-752 to G-764; I-753 to G-764; L-754 to G-764; I-755 to G-764; F-756 to G-764; S-757 to G-764; K-758 to G-764; and/or K-759 to G-764; of the amino acid sequence of SEQ ID NO:2.

[0086] As mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind EF or LF) may still be retained. For example, the ability of the shortened PA polypeptide to induce and/or bind to antibodies which recognize the complete or mature forms of the PA polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a PA polypeptide with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six PA amino acid residues may often evoke an immune response.

[0087] Accordingly, the present invention further provides antibodies that bind polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the PA polypeptide sequence of SEQ ID NO:2 up to the arginine residue at position number 36. In particular, the present invention provides antibodies that bind polypeptides comprising the amino acid sequence of residues 30-m^1 of SEQ ID NO:2, where m^1 is an integer from 36 to 763 corresponding to the position of the amino acid residue in SEQ ID NO:2.

[0088] More in particular, the invention provides antibodies that bind polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues E-30 to I-763; E-30 to E-762; E-30 to Y-761; E-30 to G-760; E-30 to K-759; E-30 to K-758; E-30 to S-757; E-30 to F-756; E-30 to I-755; E-30 to L-754; E-30 to I-753; E-30 to K-752; E-30 to K-751; E-30 to I-750; E-30 to G-749; E-30 to N-748; E-30 to T-747; E-30 to S-746; E-30 to T-745; E-30 to D-744; E-30 to G-743; E-30 to N-742; E-30 to E-741; E-30 to S-740; E-30 to P-739; E-30 to N-738; E-30 to I-737; E-30 to I-736; E-30 to T-735; E-30 to N-734; E-30 to E-733; E-30 to K-732; E-30 to T-731; E-30 to V-730; E-30 to A-729; E-30 to Y-728; E-30 to V-727; E-30 to N-726; E-30 to V-725; E-30 to K-724; E-30 to Y-723; E-30 to N-722; E-30 to P-721; E-30 to N-720; E-30 to S-719; E-30 to I-718; E-30 to Y-717; E-30 to L-716; E-30 to P-715; E-30 to L-714; E-30 to K-713; E-30 to D-712; E-30 to N-711; E-30 to Y-710; E-30 to K-709; E-30 to K-708; E-30 to F-707; E-30 to D-706; E-30 to I-705; E-30 to F-704; E-30 to T-703; E-30 to K-702; E-30 to G-701; E-30 to D-700; E-30 to Q-699; E-30 to R-698; E-30 to L-697; E-30 to S-696; E-30 to S-695; E-30 to I-694; E-30 to N-693; E-30 to L-692; E-30 to M-691; E-30 to D-690; E-30 to Y-689; E-30 to R-688; E-30 to D-687; E-30 to N-686; E-30 to I-685; E-30 to V-684; E-30 to E-683; E-30 to K-682; E-30 to L-681; E-30 to G-680; E-30 to E-679; E-30 to T-678; E-30 to D-677; E-30 to E-676; E-30 to I-675; E-30 to E-674; E-30 to V-673; E-30 to I-672; E-30 to Y-671; E-30 to G-670; E-30 to S-669; E-30 to L-668; E-30 to I-667; E-30 to K-666; E-30 to R-665; E-30 to I-664; E-30 to D-663; E-30 to K-662; E-30 to D-661; E-30 to I-660; E-30 to N-659; E-30 to L-658; E-30 to L-657; E-30 to L-656; E-30 to G-655; E-30 to E-654; E-30 to T-653; E-30 to S-652; E-30 to S-651; E-30 to N-650; E-30 to I-649; E-30 to V-648; E-30 to E-647; E-30 to R-646; E-30 to H-645; E-30 to A-644; E-30 to E-643; E-30 to K-642; E-30 to V-641; E-30 to V-640; E-30 to S-639; E-30 to E-638; E-30 to D-637; E-30 to A-636; E-30 to G-635; E-30 to V-634; E-30 to A-633; E-30 to I-632; E-30 to N-631; E-30 to N-630; E-30 to R-629; E-30 to D-628; E-30 to Y-627; E-30 to H-626; E-30 to F-625; E-30 to R-624; E-30 to K-623; E-30 to D-622; E-30 to R-621; E-30 to I-620; E-30 to L-619; E-30 to I-618; E-30 to N-617; E-30 to M-616; E-30 to K-615; E-30 to A-614; E-30 to N-613; E-30 to L-612; E-30 to K-611; E-30 to I-610; E-30 to K-609; E-30 to D-608; E-30 to L-607; E-30 to V-606; E-30 to T-605; E-30 to Y-604; E-30 to I-603; E-30 to N-602; E-30 to T-601; E-30 to A-600; E-30 to N-599; E-30 to L-598; E-30 to E-597; E-30 to A-596; E-30 to L-595; E-30 to Q-594; E-30 to N-593; E-30 to K-592; E-30 to I-591; E-30 to N-590; E-30 to Q-589; E-30 to S-588; E-30 to T-587; E-30 to Q-586; E-30 to Q-585; E-30 to D-584; E-

30 to F-583; E-30 to N-582; E-30 to F-581; E-30 to D-580; E-30 to F-579; E-30 to E-578; E-30 to T-577; E-30 to I-576; E-30 to D-575; E-30 to K-574; E-30 to G-573; E-30 to Q-572; E-30 to Y-571; E-30 to Q-570; E-30 to L-569; E-30 to N-568; E-30 to G-567; E-30 to N-566; E-30 to P-565; E-30 to E-564; E-30 to N-563; E-30 to F-562; E-30 to G-561; E-30 to F-560; E-30 to A-559; E-30 to I-558; E-30 to K-557; E-30 to L-556; E-30 to A-555; E-30 to E-554; E-30 to K-553; E-30 to L-552; E-30 to T-551; E-30 to M-550; E-30 to D-549; E-30 to P-548; E-30 to K-547; E-30 to T-546; E-30 to T-545; E-30 to E-544; E-30 to L-543; E-30 to P-542; E-30 to D-541; E-30 to S-540; E-30 to P-539; E-30 to N-538; E-30 to V-537; E-30 to A-536; E-30 to A-535; E-30 to I-534; E-30 to R-533; E-30 to R-532; E-30 to E-531; E-30 to V-530; E-30 to L-529; E-30 to N-528; E-30 to L-527; E-30 to D-526; E-30 to K-525; E-30 to G-524; E-30 to N-523; E-30 to F-522; E-30 to I-521; E-30 to I-520; E-30 to R-519; E-30 to A-518; E-30 to T-517; E-30 to T-516; E-30 to E-515; E-30 to Q-514; E-30 to I-513; E-30 to Q-512; E-30 to P-511; E-30 to L-510; E-30 to V-509; E-30 to E-508; E-30 to S-507; E-30 to W-506; E-30 to N-505; E-30 to S-504; E-30 to G-503; E-30 to T-502; E-30 to D-501; E-30 to V-500; E-30 to R-499; E-30 to V-498; E-30 to R-497; E-30 to G-496; E-30 to N-495; E-30 to E-494; E-30 to F-493; E-30 to N-492; E-30 to Y-491; E-30 to T-490; E-30 to A-489; E-30 to I-488; E-30 to N-487; E-30 to G-486; E-30 to Y-485; E-30 to V-484; E-30 to Q-483; E-30 to D-482; E-30 to T-481; E-30 to D-480; E-30 to L-479; E-30 to R-478; E-30 to L-477; E-30 to Q-476; E-30 to K-475; E-30 to T-474; E-30 to K-473; E-30 to E-472; E-30 to L-471; E-30 to E-470; E-30 to L-469; E-30 to F-468; E-30 to Q-467; E-30 to N-466; E-30 to Y-465; E-30 to N-464; E-30 to M-463; E-30 to T-462; E-30 to I-461; E-30 to P-460; E-30 to T-459; E-30 to S-458; E-30 to S-457; E-30 to F-456; E-30 to D-455; E-30 to D-454; E-30 to Q-453; E-30 to A-452; E-30 to N-451; E-30 to L-450; E-30 to A-449; E-30 to I-448; E-30 to P-447; E-30 to A-446; E-30 to L-445; E-30 to N-444; E-30 to K-443; E-30 to S-442; E-30 to P-441; E-30 to Y-440; E-30 to Y-439; E-30 to N-438; E-30 to N-437; E-30 to P-436; E-30 to A-435; E-30 to L-434; E-30 to I-433; E-30 to Q-432; E-30 to S-431; E-30 to L-430; E-30 to Q-429; E-30 to N-428; E-30 to E-427; E-30 to K-426; E-30 to A-425; E-30 to K-424; E-30 to I-423; E-30 to T-422; E-30 to A-421; E-30 to L-420; E-30 to T-419; E-30 to Q-418; E-30 to N-417; E-30 to K-416; E-30 to G-415; E-30 to L-414; E-30 to V-413; E-30 to L-412; E-30 to S-411; E-30 to T-410; E-30 to T-409; E-30 to P-408; E-30 to L-407; E-30 to V-406; E-30 to N-405; E-30 to Y-404; E-30 to I-403; E-30 to P-402; E-30 to A-401; E-30 to T-400; E-30 to G-399; E-30 to T-398; E-30 to N-397; E-30 to V-396; E-30 to Y-395; E-30 to R-394; E-30 to I-

393; E-30 to N-392; E-30 to A-391; E-30 to N-390; E-30 to L-389; E-30 to R-388; E-30 to A-387; E-30 to T-386; E-30 to D-385; E-30 to A-384; E-30 to T-383; E-30 to N-382; E-30 to L-381; E-30 to G-380; E-30 to M-379; E-30 to T-378; E-30 to E-377; E-30 to A-376; E-30 to W-375; E-30 to T-374; E-30 to R-373; E-30 to E-372; E-30 to G-371; E-30 to A-370; E-30 to L-369; E-30 to S-368; E-30 to L-367; E-30 to S-366; E-30 to H-365; E-30 to D-364; E-30 to I-363; E-30 to A-362; E-30 to V-361; E-30 to T-360; E-30 to S-359; E-30 to S-358; E-30 to N-357; E-30 to S-356; E-30 to N-355; E-30 to S-354; E-30 to F-353; E-30 to G-352; E-30 to A-351; E-30 to S-350; E-30 to V-349; E-30 to S-348; E-30 to G-347; E-30 to G-346; E-30 to I-345; E-30 to D-344; E-30 to F-343; E-30 to F-342; E-30 to S-341; E-30 to A-340; E-30 to H-339; E-30 to V-338; E-30 to E-337; E-30 to A-336; E-30 to N-335; E-30 to G-334; E-30 to H-333; E-30 to V-332; E-30 to E-331; E-30 to S-330; E-30 to T-329; E-30 to H-328; E-30 to T-327; E-30 to R-326; E-30 to S-325; E-30 to T-324; E-30 to S-323; E-30 to T-322; E-30 to N-321; E-30 to K-320; E-30 to S-319; E-30 to I-318; E-30 to T-317; E-30 to R-316; E-30 to T-315; E-30 to Q-314; E-30 to S-313; E-30 to D-312; E-30 to T-311; E-30 to N-310; E-30 to Q-309; E-30 to T-308; E-30 to S-307; E-30 to Q-306; E-30 to D-305; E-30 to E-304; E-30 to N-303; E-30 to K-302; E-30 to S-301; E-30 to L-300; E-30 to I-299; E-30 to I-298; E-30 to N-297; E-30 to E-296; E-30 to M-295; E-30 to D-294; E-30 to V-293; E-30 to H-292; E-30 to V-291; E-30 to I-290; E-30 to P-289; E-30 to Y-288; E-30 to A-287; E-30 to A-286; E-30 to V-285; E-30 to L-284; E-30 to P-283; E-30 to H-282; E-30 to R-281; E-30 to A-280; E-30 to E-279; E-30 to P-278; E-30 to S-277; E-30 to V-276; E-30 to N-275; E-30 to K-274; E-30 to D-273; E-30 to I-272; E-30 to R-271; E-30 to G-270; E-30 to T-269; E-30 to V-268; E-30 to K-267; E-30 to E-266; E-30 to F-265; E-30 to D-264; E-30 to S-263; E-30 to Y-262; E-30 to P-261; E-30 to D-260; E-30 to S-259; E-30 to A-258; E-30 to T-257; E-30 to S-256; E-30 to W-255; E-30 to K-254; E-30 to E-253; E-30 to P-252; E-30 to S-251; E-30 to S-250; E-30 to K-249; E-30 to Y-248; E-30 to K-247; E-30 to T-246; E-30 to L-245; E-30 to G-244; E-30 to K-243; E-30 to K-242; E-30 to E-241; E-30 to H-240; E-30 to I-239; E-30 to N-238; E-30 to S-237; E-30 to I-236; E-30 to W-235; E-30 to P-234; E-30 to S-233; E-30 to L-232; E-30 to F-231; E-30 to T-230; E-30 to R-229; E-30 to K-228; E-30 to N-227; E-30 to K-226; E-30 to V-225; E-30 to D-224; E-30 to V-223; E-30 to T-222; E-30 to Y-221; E-30 to G-220; E-30 to E-219; E-30 to V-218; E-30 to E-217; E-30 to L-216; E-30 to S-215; E-30 to D-214; E-30 to P-213; E-30 to I-212; E-30 to G-211; E-30 to D-210; E-30 to N-209; E-30 to D-208; E-30 to R-207; E-30 to D-206; E-30 to P-205; E-30 to V-204; E-30 to T-203; E-

30 to P-202; E-30 to G-201; E-30 to A-200; E-30 to S-199; E-30 to T-198; E-30 to S-197; E-30 to R-196; E-30 to K-195; E-30 to K-194; E-30 to R-193; E-30 to S-192; E-30 to N-191; E-30 to S-190; E-30 to S-189; E-30 to K-188; E-30 to Q-187; E-30 to K-186; E-30 to L-185; E-30 to E-184; E-30 to P-183; E-30 to L-182; E-30 to Q-181; E-30 to L-180; E-30 to N-179; E-30 to D-178; E-30 to S-177; E-30 to S-176; E-30 to I-175; E-30 to V-174; E-30 to E-173; E-30 to K-172; E-30 to K-171; E-30 to N-170; E-30 to Q-169; E-30 to S-168; E-30 to D-167; E-30 to T-166; E-30 to W-165; E-30 to Y-164; E-30 to L-163; E-30 to K-162; E-30 to F-161; E-30 to D-160; E-30 to L-159; E-30 to G-158; E-30 to K-157; E-30 to E-156; E-30 to T-155; E-30 to P-154; E-30 to N-153; E-30 to E-152; E-30 to R-151; E-30 to Q-150; E-30 to Y-149; E-30 to Q-148; E-30 to I-147; E-30 to K-146; E-30 to I-145; E-30 to Q-144; E-30 to Y-143; E-30 to L-142; E-30 to R-141; E-30 to G-140; E-30 to K-139; E-30 to E-138; E-30 to L-137; E-30 to R-136; E-30 to I-135; E-30 to K-134; E-30 to N-133; E-30 to S-132; E-30 to N-131; E-30 to S-130; E-30 to A-129; E-30 to K-128; E-30 to N-127; E-30 to I-126; E-30 to V-125; E-30 to E-124; E-30 to Q-123; E-30 to D-122; E-30 to D-121; E-30 to V-120; E-30 to W-119; E-30 to M-118; E-30 to T-117; E-30 to V-116; E-30 to H-115; E-30 to N-114; E-30 to D-113; E-30 to A-112; E-30 to S-111; E-30 to T-110; E-30 to A-109; E-30 to F-108; E-30 to T-107; E-30 to Y-106; E-30 to E-105; E-30 to D-104; E-30 to S-103; E-30 to K-102; E-30 to K-101; E-30 to V-100; E-30 to K-99; E-30 to I-98; E-30 to F-97; E-30 to G-96; E-30 to S-95; E-30 to W-94; E-30 to I-93; E-30 to A-92; E-30 to S-91; E-30 to Q-90; E-30 to F-89; E-30 to Y-88; E-30 to Q-87; E-30 to N-86; E-30 to E-85; E-30 to S-84; E-30 to P-83; E-30 to I-82; E-30 to N-81; E-30 to E-80; E-30 to L-79; E-30 to E-78; E-30 to S-77; E-30 to S-76; E-30 to P-75; E-30 to I-74; E-30 to S-73; E-30 to L-72; E-30 to D-71; E-30 to G-70; E-30 to T-69; E-30 to T-68; E-30 to S-67; E-30 to S-66; E-30 to T-65; E-30 to V-64; E-30 to V-63; E-30 to M-62; E-30 to P-61; E-30 to A-60; E-30 to Q-59; E-30 to F-58; E-30 to N-57; E-30 to L-56; E-30 to D-55; E-30 to S-54; E-30 to F-53; E-30 to Y-52; E-30 to Y-51; E-30 to G-50; E-30 to L-49; E-30 to L-48; E-30 to G-47; E-30 to Q-46; E-30 to S-45; E-30 to S-44; E-30 to S-43; E-30 to E-42; E-30 to S-41; E-30 to E-40; E-30 to N-39; E-30 to L-38; E-30 to L-37; and/or E-30 to R-36 of the amino acid sequence of SEQ ID NO:2.

[0089] The invention also provides antibodies that bind polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of a PA polypeptide, which may be described generally as having residues n^1- m^1 of SEQ ID NO:2, where n^1 and m^1 are integers as described above.

[0090] It will be recognized in the art that some amino acid sequence of PA can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. Such areas will usually comprise residues which make up the ligand binding site or the death domain, or which form tertiary structures which affect these domains.

[0091] Thus, the invention further includes antibodies that bind variations of the PA protein which show substantial PA protein activity or which include regions of PA such as the protein fragments discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitution. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U. *et al., Science 247:*1306-1310 (1990).

[0092] Thus, antibodies of the present invention may bind a fragment, derivative, or analog of the polypeptide of SEQ ID NO:2. Such fragments, variants or derivatives may be (i) one in which at least one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue(s), and more preferably at least one but less than ten conserved amino acid residues) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

[0093] Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the PA protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al., Clin Exp. Immunol.*

2:331-340 (1967); Robbins *et al., Diabetes 36*:838-845 (1987); Cleland *et al. Crit. Rev. Therapeutic Drug Carrier Systems 10*:307-377 (1993)).

[0094] The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade *et al., Nature 361*:266-268 (1993) describes certain mutations resulting in selective binding of TNF-alpha to only one of the two known types of TNF receptors. Thus, the antibodies of the present invention may bind a PA protein that contains one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

[0095] As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 3).

TABLE 3. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

[0096] In specific embodiments, the number of substitutions, additions or deletions in the amino acid sequence of SEQ ID NO:2 and/or any of the polypeptides or polypeptide

fragments described herein is 75, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 30-20, 20-15, 20-10, 15-10, 10-1, 5-10, 1-5, 1-3 or 1-2.

[0097] In specific embodiments, the antibodies of the invention bind PA polypeptides or fragments or variants thereof that contains any one or more of the following conservative mutations in PA: M1 replaced with A, G, I, L, S, T, or V; K2 replaced with H, or R; K3 replaced with H, or R; R4 replaced with H, or K; K5 replaced with H, or R; V6 replaced with A, G, I, L, S, T, or M; L7 replaced with A, G, I, S, T, M, or V; I8 replaced with A, G, L, S, T, M, or V; L10 replaced with A, G, I, S, T, M, or V; M11 replaced with A, G, I, L, S, T, or V; A12 replaced with G, I, L, S, T, M, or V; L13 replaced with A, G, I, S, T, M, or V; S14 replaced with A, G, I, L, T, M, or V; T15 replaced with A, G, I, L, S, M, or V; I16 replaced with A, G, L, S, T, M, or V; L17 replaced with A, G, I, S, T, M, or V; V18 replaced with A, G, I, L, S, T, or M; S19 replaced with A, G, I, L, T, M, or V; S20 replaced with A, G, I, L, T, M, or V; T21 replaced with A, G, I, L, S, M, or V; G22 replaced with A, I, L, S, T, M, or V; N23 replaced with Q; L24 replaced with A, G, I, S, T, M, or V; E25 replaced with D; V26 replaced with A, G, I, L, S, T, or M; I27 replaced with A, G, L, S, T, M, or V; Q28 replaced with N; A29 replaced with G, I, L, S, T, M, or V; E30 replaced with D; V31 replaced with A, G, I, L, S, T, or M; K32 replaced with H, or R; Q33 replaced with N; E34 replaced with D; N35 replaced with Q; R36 replaced with H, or K; L37 replaced with A, G, I, S, T, M, or V; L38 replaced with A, G, I, S, T, M, or V; N39 replaced with Q; E40 replaced with D; S41 replaced with A, G, I, L, T, M, or V; E42 replaced with D; S43 replaced with A, G, I, L, T, M, or V; S44 replaced with A, G, I, L, T, M, or V; S45 replaced with A, G, I, L, T, M, or V; Q46 replaced with N; G47 replaced with A, I, L, S, T, M, or V; L48 replaced with A, G, I, S, T, M, or V; L49 replaced with A, G, I, S, T, M, or V; G50 replaced with A, I, L, S, T, M, or V; Y51 replaced with F, or W; Y52 replaced with F, or W; F53 replaced with W, or Y; S54 replaced with A, G, I, L, T, M, or V; D55 replaced with E; L56 replaced with A, G, I, S, T, M, or V; N57 replaced with Q; F58 replaced with W, or Y; Q59 replaced with N; A60 replaced with G, I, L, S, T, M, or V; M62 replaced with A, G, I, L, S, T, or V; V63 replaced with A, G, I, L, S, T, or M; V64 replaced with A, G, I, L, S, T, or M; T65 replaced with A, G, I, L, S, M, or V; S66 replaced with A, G, I, L, T, M, or V; S67 replaced with A, G, I, L, T, M, or V; T68 replaced with A, G, I, L, S, M, or V; T69 replaced with A, G, I, L, S, M, or V; G70 replaced with A, I, L, S, T, M, or V; D71 replaced with E; L72 replaced with A, G, I, S, T,

M, or V; S73 replaced with A, G, I, L, T, M, or V; I74 replaced with A, G, L, S, T, M, or V; S76 replaced with A, G, I, L, T, M, or V; S77 replaced with A, G, I, L, T, M, or V; E78 replaced with D; L79 replaced with A, G, I, S, T, M, or V; E80 replaced with D; N81 replaced with Q; I82 replaced with A, G, L, S, T, M, or V; S84 replaced with A, G, I, L, T, M, or V; E85 replaced with D; N86 replaced with Q; Q87 replaced with N; Y88 replaced with F, or W; F89 replaced with W, or Y; Q90 replaced with N; S91 replaced with A, G, I, L, T, M, or V; A92 replaced with G, I, L, S, T, M, or V; I93 replaced with A, G, L, S, T, M, or V; W94 replaced with F, or Y; S95 replaced with A, G, I, L, T, M, or V; G96 replaced with A, I, L, S, T, M, or V; F97 replaced with W, or Y; I98 replaced with A, G, L, S, T, M, or V; K99 replaced with H, or R; V100 replaced with A, G, I, L, S, T, or M; K101 replaced with H, or R; K102 replaced with H, or R; S103 replaced with A, G, I, L, T, M, or V; D104 replaced with E; E105 replaced with D; Y106 replaced with F, or W; T107 replaced with A, G, I, L, S, M, or V; F108 replaced with W, or Y; A109 replaced with G, I, L, S, T, M, or V; T110 replaced with A, G, I, L, S, M, or V; S111 replaced with A, G, I, L, T, M, or V; A112 replaced with G, I, L, S, T, M, or V; D113 replaced with E; N114 replaced with Q; H115 replaced with K, or R; V116 replaced with A, G, I, L, S, T, or M; T117 replaced with A, G, I, L, S, M, or V; M118 replaced with A, G, I, L, S, T, or V; W119 replaced with F, or Y; V120 replaced with A, G, I, L, S, T, or M; D121 replaced with E; D122 replaced with E; Q123 replaced with N; E124 replaced with D; V125 replaced with A, G, I, L, S, T, or M; I126 replaced with A, G, L, S, T, M, or V; N127 replaced with Q; K128 replaced with H, or R; A129 replaced with G, I, L, S, T, M, or V; S130 replaced with A, G, I, L, T, M, or V; N131 replaced with Q; S132 replaced with A, G, I, L, T, M, or V; N133 replaced with Q; K134 replaced with H, or R; I135 replaced with A, G, L, S, T, M, or V; R136 replaced with H, or K; L137 replaced with A, G, I, S, T, M, or V; E138 replaced with D; K139 replaced with H, or R; G140 replaced with A, I, L, S, T, M, or V; R141 replaced with H, or K; L142 replaced with A, G, I, S, T, M, or V; Y143 replaced with F, or W; Q144 replaced with N; I145 replaced with A, G, L, S, T, M, or V; K146 replaced with H, or R; I147 replaced with A, G, L, S, T, M, or V; Q148 replaced with N; Y149 replaced with F, or W; Q150 replaced with N; R151 replaced with H, or K; E152 replaced with D; N153 replaced with Q; T155 replaced with A, G, I, L, S, M, or V; E156 replaced with D; K157 replaced with H, or R; G158 replaced with A, I, L, S, T, M, or V; L159 replaced with A, G, I, S, T, M, or V; D160 replaced with E; F161 replaced with W, or Y; K162 replaced with H, or R; L163 replaced with A, G, I, S, T, M,

or V; Y164 replaced with F, or W; W165 replaced with F, or Y; T166 replaced with A, G, I, L, S, M, or V; D167 replaced with E; S168 replaced with A, G, I, L, T, M, or V; Q169 replaced with N; N170 replaced with Q; K171 replaced with H, or R; K172 replaced with H, or R; E173 replaced with D; V174 replaced with A, G, I, L, S, T, or M; I175 replaced with A, G, L, S, T, M, or V; S176 replaced with A, G, I, L, T, M, or V; S177 replaced with A, G, I, L, T, M, or V; D178 replaced with E; N179 replaced with Q; L180 replaced with A, G, I, S, T, M, or V; Q181 replaced with N; L182 replaced with A, G, I, S, T, M, or V; E184 replaced with D; L185 replaced with A, G, I, S, T, M, or V; K186 replaced with H, or R; Q187 replaced with N; K188 replaced with H, or R; S189 replaced with A, G, I, L, T, M, or V; S190 replaced with A, G, I, L, T, M, or V; N191 replaced with Q; S192 replaced with A, G, I, L, T, M, or V; R193 replaced with H, or K; K194 replaced with H, or R; K195 replaced with H, or R; R196 replaced with H, or K; S197 replaced with A, G, I, L, T, M, or V; T198 replaced with A, G, I, L, S, M, or V; S199 replaced with A, G, I, L, T, M, or V; A200 replaced with G, I, L, S, T, M, or V; G201 replaced with A, I, L, S, T, M, or V; T203 replaced with A, G, I, L, S, M, or V; V204 replaced with A, G, I, L, S, T, or M; D206 replaced with E; R207 replaced with H, or K; D208 replaced with E; N209 replaced with Q; D210 replaced with E; G211 replaced with A, I, L, S, T, M, or V; I212 replaced with A, G, L, S, T, M, or V; D214 replaced with E; S215 replaced with A, G, I, L, T, M, or V; L216 replaced with A, G, I, S, T, M, or V; E217 replaced with D; V218 replaced with A, G, I, L, S, T, or M; E219 replaced with D; G220 replaced with A, I, L, S, T, M, or V; Y221 replaced with F, or W; T222 replaced with A, G, I, L, S, M, or V; V223 replaced with A, G, I, L, S, T, or M; D224 replaced with E; V225 replaced with A, G, I, L, S, T, or M; K226 replaced with H, or R; N227 replaced with Q; K228 replaced with H, or R; R229 replaced with H, or K; T230 replaced with A, G, I, L, S, M, or V; F231 replaced with W, or Y; L232 replaced with A, G, I, S, T, M, or V; S233 replaced with A, G, I, L, T, M, or V; W235 replaced with F, or Y; I236 replaced with A, G, L, S, T, M, or V; S237 replaced with A, G, I, L, T, M, or V; N238 replaced with Q; I239 replaced with A, G, L, S, T, M, or V; H240 replaced with K, or R; E241 replaced with D; K242 replaced with H, or R; K243 replaced with H, or R; G244 replaced with A, I, L, S, T, M, or V; L245 replaced with A, G, I, S, T, M, or V; T246 replaced with A, G, I, L, S, M, or V; K247 replaced with H, or R; Y248 replaced with F, or W; K249 replaced with H, or R; S250 replaced with A, G, I, L, T, M, or V; S251 replaced with A, G, I, L, T, M, or V; E253 replaced with D; K254 replaced with H, or R; W255 replaced with F, or Y; S256 replaced with A, G, I, L,

T, M, or V; T257 replaced with A, G, I, L, S, M, or V; A258 replaced with G, I, L, S, T, M, or V; S259 replaced with A, G, I, L, T, M, or V; D260 replaced with E; Y262 replaced with F, or W; S263 replaced with A, G, I, L, T, M, or V; D264 replaced with E; F265 replaced with W, or Y; E266 replaced with D; K267 replaced with H, or R; V268 replaced with A, G, I, L, S, T, or M; T269 replaced with A, G, I, L, S, M, or V; G270 replaced with A, I, L, S, T, M, or V; R271 replaced with H, or K; I272 replaced with A, G, L, S, T, M, or V; D273 replaced with E; K274 replaced with H, or R; N275 replaced with Q; V276 replaced with A, G, I, L, S, T, or M; S277 replaced with A, G, I, L, T, M, or V; E279 replaced with D; A280 replaced with G, I, L, S, T, M, or V; R281 replaced with H, or K; H282 replaced with K, or R; L284 replaced with A, G, I, S, T, M, or V; V285 replaced with A, G, I, L, S, T, or M; A286 replaced with G, I, L, S, T, M, or V; A287 replaced with G, I, L, S, T, M, or V; Y288 replaced with F, or W; I290 replaced with A, G, L, S, T, M, or V; V291 replaced with A, G, I, L, S, T, or M; H292 replaced with K, or R; V293 replaced with A, G, I, L, S, T, or M; D294 replaced with E; M295 replaced with A, G, I, L, S, T, or V; E296 replaced with D; N297 replaced with Q; I298 replaced with A, G, L, S, T, M, or V; I299 replaced with A, G, L, S, T, M, or V; L300 replaced with A, G, I, S, T, M, or V; S301 replaced with A, G, I, L, T, M, or V; K302 replaced with H, or R; N303 replaced with Q; E304 replaced with D; D305 replaced with E; Q306 replaced with N; S307 replaced with A, G, I, L, T, M, or V; T308 replaced with A, G, I, L, S, M, or V; Q309 replaced with N; N310 replaced with Q; T311 replaced with A, G, I, L, S, M, or V; D312 replaced with E; S313 replaced with A, G, I, L, T, M, or V; Q314 replaced with N; T315 replaced with A, G, I, L, S, M, or V; R316 replaced with H, or K; T317 replaced with A, G, I, L, S, M, or V; I318 replaced with A, G, L, S, T, M, or V; S319 replaced with A, G, I, L, T, M, or V; K320 replaced with H, or R; N321 replaced with Q; T322 replaced with A, G, I, L, S, M, or V; S323 replaced with A, G, I, L, T, M, or V; T324 replaced with A, G, I, L, S, M, or V; S325 replaced with A, G, I, L, T, M, or V; R326 replaced with H, or K; T327 replaced with A, G, I, L, S, M, or V; H328 replaced with K, or R; T329 replaced with A, G, I, L, S, M, or V; S330 replaced with A, G, I, L, T, M, or V; E331 replaced with D; V332 replaced with A, G, I, L, S, T, or M; H333 replaced with K, or R; G334 replaced with A, I, L, S, T, M, or V; N335 replaced with Q; A336 replaced with G, I, L, S, T, M, or V; E337 replaced with D; V338 replaced with A, G, I, L, S, T, or M; H339 replaced with K, or R; A340 replaced with G, I, L, S, T, M, or V; S341 replaced with A, G, I, L, T, M, or V; F342 replaced with W, or Y; F343 replaced with W, or Y;

D344 replaced with E; I345 replaced with A, G, L, S, T, M, or V; G346 replaced with A, I, L, S, T, M, or V; G347 replaced with A, I, L, S, T, M, or V; S348 replaced with A, G, I, L, T, M, or V; V349 replaced with A, G, I, L, S, T, or M; S350 replaced with A, G, I, L, T, M, or V; A351 replaced with G, I, L, S, T, M, or V; G352 replaced with A, I, L, S, T, M, or V; F353 replaced with W, or Y; S354 replaced with A, G, I, L, T, M, or V; N355 replaced with Q; S356 replaced with A, G, I, L, T, M, or V; N357 replaced with Q; S358 replaced with A, G, I, L, T, M, or V; S359 replaced with A, G, I, L, T, M, or V; T360 replaced with A, G, I, L, S, M, or V; V361 replaced with A, G, I, L, S, T, or M; A362 replaced with G, I, L, S, T, M, or V; I363 replaced with A, G, L, S, T, M, or V; D364 replaced with E; H365 replaced with K, or R; S366 replaced with A, G, I, L, T, M, or V; L367 replaced with A, G, I, S, T, M, or V; S368 replaced with A, G, I, L, T, M, or V; L369 replaced with A, G, I, S, T, M, or V; A370 replaced with G, I, L, S, T, M, or V; G371 replaced with A, I, L, S, T, M, or V; E372 replaced with D; R373 replaced with H, or K; T374 replaced with A, G, I, L, S, M, or V; W375 replaced with F, or Y; A376 replaced with G, I, L, S, T, M, or V; E377 replaced with D; T378 replaced with A, G, I, L, S, M, or V; M379 replaced with A, G, I, L, S, T, or V; G380 replaced with A, I, L, S, T, M, or V; L381 replaced with A, G, I, S, T, M, or V; N382 replaced with Q; T383 replaced with A, G, I, L, S, M, or V; A384 replaced with G, I, L, S, T, M, or V; D385 replaced with E; T386 replaced with A, G, I, L, S, M, or V; A387 replaced with G, I, L, S, T, M, or V; R388 replaced with H, or K; L389 replaced with A, G, I, S, T, M, or V; N390 replaced with Q; A391 replaced with G, I, L, S, T, M, or V; N392 replaced with Q; I393 replaced with A, G, L, S, T, M, or V; R394 replaced with H, or K; Y395 replaced with F, or W; V396 replaced with A, G, I, L, S, T, or M; N397 replaced with Q; T398 replaced with A, G, I, L, S, M, or V; G399 replaced with A, I, L, S, T, M, or V; T400 replaced with A, G, I, L, S, M, or V; A401 replaced with G, I, L, S, T, M, or V; I403 replaced with A, G, L, S, T, M, or V; Y404 replaced with F, or W; N405 replaced with Q; V406 replaced with A, G, I, L, S, T, or M; L407 replaced with A, G, I, S, T, M, or V; T409 replaced with A, G, I, L, S, M, or V; T410 replaced with A, G, I, L, S, M, or V; S411 replaced with A, G, I, L, T, M, or V; L412 replaced with A, G, I, S, T, M, or V; V413 replaced with A, G, I, L, S, T, or M; L414 replaced with A, G, I, S, T, M, or V; G415 replaced with A, I, L, S, T, M, or V; K416 replaced with H, or R; N417 replaced with Q; Q418 replaced with N; T419 replaced with A, G, I, L, S, M, or V; L420 replaced with A, G, I, S, T, M, or V; A421 replaced with G, I, L, S, T, M, or V; T422 replaced with A, G, I, L, S, M, or V; I423 replaced with A, G,

L, S, T, M, or V; K424 replaced with H, or R; A425 replaced with G, I, L, S, T, M, or V; K426 replaced with H, or R; E427 replaced with D; N428 replaced with Q; Q429 replaced with N; L430 replaced with A, G, I, S, T, M, or V; S431 replaced with A, G, I, L, T, M, or V; Q432 replaced with N; I433 replaced with A, G, L, S, T, M, or V; L434 replaced with A, G, I, S, T, M, or V; A435 replaced with G, I, L, S, T, M, or V; N437 replaced with Q; N438 replaced with Q; Y439 replaced with F, or W; Y440 replaced with F, or W; S442 replaced with A, G, I, L, T, M, or V; K443 replaced with H, or R; N444 replaced with Q; L445 replaced with A, G, I, S, T, M, or V; A446 replaced with G, I, L, S, T, M, or V; I448 replaced with A, G, L, S, T, M, or V; A449 replaced with G, I, L, S, T, M, or V; L450 replaced with A, G, I, S, T, M, or V; N451 replaced with Q; A452 replaced with G, I, L, S, T, M, or V; Q453 replaced with N; D454 replaced with E; D455 replaced with E; F456 replaced with W, or Y; S457 replaced with A, G, I, L, T, M, or V; S458 replaced with A, G, I, L, T, M, or V; T459 replaced with A, G, I, L, S, M, or V; I461 replaced with A, G, L, S, T, M, or V; T462 replaced with A, G, I, L, S, M, or V; M463 replaced with A, G, I, L, S, T, or V; N464 replaced with Q; Y465 replaced with F, or W; N466 replaced with Q; Q467 replaced with N; F468 replaced with W, or Y; L469 replaced with A, G, I, S, T, M, or V; E470 replaced with D; L471 replaced with A, G, I, S, T, M, or V; E472 replaced with D; K473 replaced with H, or R; T474 replaced with A, G, I, L, S, M, or V; K475 replaced with H, or R; Q476 replaced with N; L477 replaced with A, G, I, S, T, M, or V; R478 replaced with H, or K; L479 replaced with A, G, I, S, T, M, or V; D480 replaced with E; T481 replaced with A, G, I, L, S, M, or V; D482 replaced with E; Q483 replaced with N; V484 replaced with A, G, I, L, S, T, or M; Y485 replaced with F, or W; G486 replaced with A, I, L, S, T, M, or V; N487 replaced with Q; I488 replaced with A, G, L, S, T, M, or V; A489 replaced with G, I, L, S, T, M, or V; T490 replaced with A, G, I, L, S, M, or V; Y491 replaced with F, or W; N492 replaced with Q; F493 replaced with W, or Y; E494 replaced with D; N495 replaced with Q; G496 replaced with A, I, L, S, T, M, or V; R497 replaced with H, or K; V498 replaced with A, G, I, L, S, T, or M; R499 replaced with H, or K; V500 replaced with A, G, I, L, S, T, or M; D501 replaced with E; T502 replaced with A, G, I, L, S, M, or V; G503 replaced with A, I, L, S, T, M, or V; S504 replaced with A, G, I, L, T, M, or V; N505 replaced with Q; W506 replaced with F, or Y; S507 replaced with A, G, I, L, T, M, or V; E508 replaced with D; V509 replaced with A, G, I, L, S, T, or M; L510 replaced with A, G, I, S, T, M, or V; Q512 replaced with N; I513 replaced with A, G, L, S, T, M, or V; Q514 replaced with N; E515 replaced with D; T516

replaced with A, G, I, L, S, M, or V; T517 replaced with A, G, I, L, S, M, or V; A518 replaced with G, I, L, S, T, M, or V; R519 replaced with H, or K; I520 replaced with A, G, L, S, T, M, or V; I521 replaced with A, G, L, S, T, M, or V; F522 replaced with W, or Y; N523 replaced with Q; G524 replaced with A, I, L, S, T, M, or V; K525 replaced with H, or R; D526 replaced with E; L527 replaced with A, G, I, S, T, M, or V; N528 replaced with Q; L529 replaced with A, G, I, S, T, M, or V; V530 replaced with A, G, I, L, S, T, or M; E531 replaced with D; R532 replaced with H, or K; R533 replaced with H, or K; I534 replaced with A, G, L, S, T, M, or V; A535 replaced with G, I, L, S, T, M, or V; A536 replaced with G, I, L, S, T, M, or V; V537 replaced with A, G, I, L, S, T, or M; N538 replaced with Q; S540 replaced with A, G, I, L, T, M, or V; D541 replaced with E; L543 replaced with A, G, I, S, T, M, or V; E544 replaced with D; T545 replaced with A, G, I, L, S, M, or V; T546 replaced with A, G, I, L, S, M, or V; K547 replaced with H, or R; D549 replaced with E; M550 replaced with A, G, I, L, S, T, or V; T551 replaced with A, G, I, L, S, M, or V; L552 replaced with A, G, I, S, T, M, or V; K553 replaced with H, or R; E554 replaced with D; A555 replaced with G, I, L, S, T, M, or V; L556 replaced with A, G, I, S, T, M, or V; K557 replaced with H, or R; I558 replaced with A, G, L, S, T, M, or V; A559 replaced with G, I, L, S, T, M, or V; F560 replaced with W, or Y; G561 replaced with A, I, L, S, T, M, or V; F562 replaced with W, or Y; N563 replaced with Q; E564 replaced with D; N566 replaced with Q; G567 replaced with A, I, L, S, T, M, or V; N568 replaced with Q; L569 replaced with A, G, I, S, T, M, or V; Q570 replaced with N; Y571 replaced with F, or W; Q572 replaced with N; G573 replaced with A, I, L, S, T, M, or V; K574 replaced with H, or R; D575 replaced with E; I576 replaced with A, G, L, S, T, M, or V; T577 replaced with A, G, I, L, S, M, or V; E578 replaced with D; F579 replaced with W, or Y; D580 replaced with E; F581 replaced with W, or Y; N582 replaced with Q; F583 replaced with W, or Y; D584 replaced with E; Q585 replaced with N; Q586 replaced with N; T587 replaced with A, G, I, L, S, M, or V; S588 replaced with A, G, I, L, T, M, or V; Q589 replaced with N; N590 replaced with Q; I591 replaced with A, G, L, S, T, M, or V; K592 replaced with H, or R; N593 replaced with Q; Q594 replaced with N; L595 replaced with A, G, I, S, T, M, or V; A596 replaced with G, I, L, S, T, M, or V; E597 replaced with D; L598 replaced with A, G, I, S, T, M, or V; N599 replaced with Q; A600 replaced with G, I, L, S, T, M, or V; T601 replaced with A, G, I, L, S, M, or V; N602 replaced with Q; I603 replaced with A, G, L, S, T, M, or V; Y604 replaced with F, or W; T605 replaced with A, G, I, L, S, M, or V; V606 replaced with A, G, I, L, S, T, or M; L607 replaced with

A, G, I, S, T, M, or V; D608 replaced with E; K609 replaced with H, or R; I610 replaced with A, G, L, S, T, M, or V; K611 replaced with H, or R; L612 replaced with A, G, I, S, T, M, or V; N613 replaced with Q; A614 replaced with G, I, L, S, T, M, or V; K615 replaced with H, or R; M616 replaced with A, G, I, L, S, T, or V; N617 replaced with Q; I618 replaced with A, G, L, S, T, M, or V; L619 replaced with A, G, I, S, T, M, or V; I620 replaced with A, G, L, S, T, M, or V; R621 replaced with H, or K; D622 replaced with E; K623 replaced with H, or R; R624 replaced with H, or K; F625 replaced with W, or Y; H626 replaced with K, or R; Y627 replaced with F, or W; D628 replaced with E; R629 replaced with H, or K; N630 replaced with Q; N631 replaced with Q; I632 replaced with A, G, L, S, T, M, or V; A633 replaced with G, I, L, S, T, M, or V; V634 replaced with A, G, I, L, S, T, or M; G635 replaced with A, I, L, S, T, M, or V; A636 replaced with G, I, L, S, T, M, or V; D637 replaced with E; E638 replaced with D; S639 replaced with A, G, I, L, T, M, or V; V640 replaced with A, G, I, L, S, T, or M; V641 replaced with A, G, I, L, S, T, or M; K642 replaced with H, or R; E643 replaced with D; A644 replaced with G, I, L, S, T, M, or V; H645 replaced with K, or R; R646 replaced with H, or K; E647 replaced with D; V648 replaced with A, G, I, L, S, T, or M; I649 replaced with A, G, L, S, T, M, or V; N650 replaced with Q; S651 replaced with A, G, I, L, T, M, or V; S652 replaced with A, G, I, L, T, M, or V; T653 replaced with A, G, I, L, S, M, or V; E654 replaced with D; G655 replaced with A, I, L, S, T, M, or V; L656 replaced with A, G, I, S, T, M, or V; L657 replaced with A, G, I, S, T, M, or V; L658 replaced with A, G, I, S, T, M, or V; N659 replaced with Q; I660 replaced with A, G, L, S, T, M, or V; D661 replaced with E; K662 replaced with H, or R; D663 replaced with E; I664 replaced with A, G, L, S, T, M, or V; R665 replaced with H, or K; K666 replaced with H, or R; I667 replaced with A, G, L, S, T, M, or V; L668 replaced with A, G, I, S, T, M, or V; S669 replaced with A, G, I, L, T, M, or V; G670 replaced with A, I, L, S, T, M, or V; Y671 replaced with F, or W; I672 replaced with A, G, L, S, T, M, or V; V673 replaced with A, G, I, L, S, T, or M; E674 replaced with D; I675 replaced with A, G, L, S, T, M, or V; E676 replaced with D; D677 replaced with E; T678 replaced with A, G, I, L, S, M, or V; E679 replaced with D; G680 replaced with A, I, L, S, T, M, or V; L681 replaced with A, G, I, S, T, M, or V; K682 replaced with H, or R; E683 replaced with D; V684 replaced with A, G, I, L, S, T, or M; I685 replaced with A, G, L, S, T, M, or V; N686 replaced with Q; D687 replaced with E; R688 replaced with H, or K; Y689 replaced with F, or W; D690 replaced with E; M691 replaced with A, G, I, L, S, T, or V; L692 replaced with A, G, I, S, T, M, or V; N693

replaced with Q; I694 replaced with A, G, L, S, T, M, or V; S695 replaced with A, G, I, L, T, M, or V; S696 replaced with A, G, I, L, T, M, or V; L697 replaced with A, G, I, S, T, M, or V; R698 replaced with H, or K; Q699 replaced with N; D700 replaced with E; G701 replaced with A, I, L, S, T, M, or V; K702 replaced with H, or R; T703 replaced with A, G, I, L, S, M, or V; F704 replaced with W, or Y; I705 replaced with A, G, L, S, T, M, or V; D706 replaced with E; F707 replaced with W, or Y; K708 replaced with H, or R; K709 replaced with H, or R; Y710 replaced with F, or W; N711 replaced with Q; D712 replaced with E; K713 replaced with H, or R; L714 replaced with A, G, I, S, T, M, or V; L716 replaced with A, G, I, S, T, M, or V; Y717 replaced with F, or W; I718 replaced with A, G, L, S, T, M, or V; S719 replaced with A, G, I, L, T, M, or V; N720 replaced with Q; N722 replaced with Q; Y723 replaced with F, or W; K724 replaced with H, or R; V725 replaced with A, G, I, L, S, T, or M; N726 replaced with Q; V727 replaced with A, G, I, L, S, T, or M; Y728 replaced with F, or W; A729 replaced with G, I, L, S, T, M, or V; V730 replaced with A, G, I, L, S, T, or M; T731 replaced with A, G, I, L, S, M, or V; K732 replaced with H, or R; E733 replaced with D; N734 replaced with Q; T735 replaced with A, G, I, L, S, M, or V; I736 replaced with A, G, L, S, T, M, or V; I737 replaced with A, G, L, S, T, M, or V; N738 replaced with Q; S740 replaced with A, G, I, L, T, M, or V; E741 replaced with D; N742 replaced with Q; G743 replaced with A, I, L, S, T, M, or V; D744 replaced with E; T745 replaced with A, G, I, L, S, M, or V; S746 replaced with A, G, I, L, T, M, or V; T747 replaced with A, G, I, L, S, M, or V; N748 replaced with Q; G749 replaced with A, I, L, S, T, M, or V; I750 replaced with A, G, L, S, T, M, or V; K751 replaced with H, or R; K752 replaced with H, or R; I753 replaced with A, G, L, S, T, M, or V; L754 replaced with A, G, I, S, T, M, or V; I755 replaced with A, G, L, S, T, M, or V; F756 replaced with W, or Y; S757 replaced with A, G, I, L, T, M, or V; K758 replaced with H, or R; K759 replaced with H, or R; G760 replaced with A, I, L, S, T, M, or V; Y761 replaced with F, or W; E762 replaced with D; I763 replaced with A, G, L, S, T, M, or V; G764 replaced with A, I, L, S, T, M, or V; of SEQ ID NO:2.

[0098] In specific embodiments, the antibodies of the invention bind PA polypeptides or fragments or variants thereof, that contains any one or more of the following non-conservative mutations in PA: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K2 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K3 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R4 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K5 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P,

or C; V6 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L7 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I8 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P9 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L10 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M11 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L13 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S14 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T15 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I16 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L17 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V18 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S19 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S20 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T21 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G22 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N23 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L24 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E25 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V26 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I27 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q28 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A29 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E30 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V31 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K32 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q33 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E34 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N35 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R36 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L37 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L38 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N39 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E40 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S41 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E42 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S43 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S44 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S45 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q46 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G47 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L48 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G50 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y51 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Y52 replaced with D, E, H, K, R, N, Q, A, G, I, L,

S, T, M, V, P, or C; F53 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D55 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N57 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F58 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q59 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A60 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P61 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; M62 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V63 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V64 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S66 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S67 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T68 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T69 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G70 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D71 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L72 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S73 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I74 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P75 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S76 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S77 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E78 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L79 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E80 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N81 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I82 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P83 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S84 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E85 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N86 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q87 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y88 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F89 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q90 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A92 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I93 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W94 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S95 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G96 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F97 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I98 replaced with

D, E, H, K, R, N, Q, F, W, Y, P, or C; K99 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V100 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K101 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K102 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S103 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D104 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E105 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y106 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T107 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F108 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A109 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S111 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A112 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D113 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N114 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; H115 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V116 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T117 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M118 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W119 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V120 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D121 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D122 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q123 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E124 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V125 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I126 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N127 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K128 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A129 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S130 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N131 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S132 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N133 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K134 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I135 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R136 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L137 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E138 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K139 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G140 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R141 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L142

replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y143 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q144 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I145 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K146 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I147 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q148 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y149 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q150 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R151 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E152 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N153 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P154 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T155 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E156 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K157 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G158 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L159 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D160 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F161 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K162 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L163 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y164 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W165 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T166 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D167 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S168 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q169 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N170 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K171 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K172 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E173 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V174 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I175 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S176 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S177 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D178 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N179 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L180 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q181 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L182 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P183 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E184 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F,

W, Y, P, or C; L185 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K186 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q187 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K188 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S189 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S190 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N191 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S192 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R193 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K194 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K195 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R196 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S197 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T198 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S199 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A200 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G201 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P202 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T203 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V204 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P205 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D206 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R207 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D208 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N209 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D210 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G211 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I212 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P213 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D214 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S215 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L216 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E217 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V218 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E219 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G220 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y221 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T222 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V223 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D224 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V225 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K226 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N227 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K228 replaced with D, E, A, G, I, L, S, T, M, V, N,

Q, F, W, Y, P, or C; R229 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T230 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F231 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L232 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S233 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P234 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; W235 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I236 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N238 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I239 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H240 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E241 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K242 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K243 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G244 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L245 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T246 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K247 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y248 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K249 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S250 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S251 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P252 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E253 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K254 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; W255 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S256 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T257 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A258 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S259 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D260 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P261 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Y262 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S263 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D264 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F265 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E266 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K267 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V268 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T269 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G270 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R271 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I272 replaced with D, E, H, K, R, N, Q, F, W,

Y, P, or C; D273 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K274 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N275 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V276 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S277 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P278 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E279 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A280 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R281 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H282 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P283 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L284 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V285 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A286 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A287 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y288 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P289 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I290 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V291 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H292 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V293 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D294 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M295 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E296 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N297 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I298 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I299 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L300 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S301 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K302 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N303 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E304 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D305 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q306 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S307 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T308 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q309 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N310 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T311 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D312 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S313 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q314 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T315 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R316 replaced with D, E, A,

G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T317 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I318 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S319 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K320 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N321 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T322 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S323 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T324 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S325 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R326 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T327 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H328 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T329 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S330 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E331 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V332 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H333 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G334 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N335 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A336 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E337 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V338 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H339 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A340 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S341 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F342 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F343 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D344 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I345 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G346 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G347 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S348 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V349 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S350 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A351 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G352 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F353 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S354 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N355 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S356 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N357 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S358 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S359 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T360 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V361 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A362 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or

C; I363 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D364 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H365 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S366 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L367 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S368 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L369 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A370 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G371 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E372 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R373 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T374 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W375 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A376 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E377 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T378 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M379 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G380 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L381 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N382 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T383 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A384 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D385 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T386 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A387 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R388 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L389 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N390 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A391 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N392 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I393 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R394 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y395 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V396 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N397 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T398 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G399 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T400 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A401 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P402 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I403 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y404 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N405 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V406 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L407 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P408 replaced with D, E, H, K,

R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; T409 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T410 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S411 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L412 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V413 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L414 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G415 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K416 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N417 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q418 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T419 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L420 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A421 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T422 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I423 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K424 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A425 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K426 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E427 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N428 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q429 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L430 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S431 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q432 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I433 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L434 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A435 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P436 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N437 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N438 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y439 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Y440 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P441 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S442 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K443 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N444 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L445 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A446 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P447 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I448 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A449 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L450 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N451 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A452 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C;

Q453 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D454 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D455 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F456 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S457 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S458 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T459 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P460 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I461 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T462 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M463 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N464 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y465 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N466 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q467 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F468 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L469 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E470 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L471 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E472 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K473 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T474 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K475 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q476 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L477 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R478 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L479 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D480 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T481 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D482 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q483 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V484 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y485 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G486 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N487 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I488 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A489 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T490 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y491 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N492 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F493 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E494 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N495 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G496

replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R497 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V498 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R499 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V500 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D501 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T502 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G503 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S504 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N505 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; W506 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S507 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E508 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V509 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L510 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P511 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Q512 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I513 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q514 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E515 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T516 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T517 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A518 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R519 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I520 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I521 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F522 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N523 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G524 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K525 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D526 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L527 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N528 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L529 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V530 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E531 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R532 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R533 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I534 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A535 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A536 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V537 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N538 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P539 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S540 replaced with D, E, H, K, R, N, Q, F, W,

Y, P, or C; D541 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P542 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L543 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E544 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T545 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T546 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K547 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P548 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; D549 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M550 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T551 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L552 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K553 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E554 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A555 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L556 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K557 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I558 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A559 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F560 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G561 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F562 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N563 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E564 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P565 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N566 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G567 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N568 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L569 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q570 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y571 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q572 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G573 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K574 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D575 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I576 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T577 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E578 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F579 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D580 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F581 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N582 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F583 replaced with D, E, H, K, R, N, Q, A, G, I, L,

S, T, M, V, P, or C; D584 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q585 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q586 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T587 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S588 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q589 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N590 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I591 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K592 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N593 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q594 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L595 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A596 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E597 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L598 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N599 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A600 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T601 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N602 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I603 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y604 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T605 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V606 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L607 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D608 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K609 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I610 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K611 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L612 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N613 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A614 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K615 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M616 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N617 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I618 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L619 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I620 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R621 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D622 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K623 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R624 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F625 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; H626 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y627 replaced

with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D628 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R629 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N630 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N631 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I632 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A633 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V634 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G635 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A636 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D637 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E638 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S639 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V640 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V641 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K642 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E643 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A644 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H645 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R646 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E647 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V648 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I649 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N650 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; S651 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S652 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T653 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E654 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G655 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L656 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L657 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L658 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N659 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I660 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D661 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K662 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D663 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I664 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R665 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K666 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I667 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L668 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S669 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G670 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y671 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M,

V, P, or C; I672 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V673 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E674 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I675 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E676 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; D677 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T678 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E679 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G680 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L681 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K682 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E683 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V684 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I685 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N686 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D687 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R688 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y689 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D690 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; M691 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L692 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N693 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I694 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S695 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S696 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L697 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R698 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q699 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D700 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G701 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K702 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T703 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F704 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I705 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D706 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F707 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K708 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K709 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y710 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N711 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; D712 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K713 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L714 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P715 replaced with D, E, H, K,

R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L716 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y717 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I718 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S719 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N720 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P721 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; N722 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y723 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K724 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; V725 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N726 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V727 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y728 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A729 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V730 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T731 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K732 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E733 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N734 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T735 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I736 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I737 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N738 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P739 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; S740 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E741 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N742 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G743 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D744 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T745 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S746 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T747 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N748 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; G749 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I750 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K751 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K752 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I753 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L754 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I755 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F756 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S757 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K758 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K759 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y,

P, or C; G760 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y761 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; E762 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I763 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G764 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; of SEQ ID NO:2.

[0099] Amino acids in the PA protein that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science 244*:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or protein multimerization, pore formation, and toxin translocation. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos *et al. Science 255*:306-312 (1992)). In preferred embodiments, antibodies of the present invention bind regions of PA that are essential for PA function. In other preferred embodiments, antibodies of the present invention bind regions of PA that are essential for PA function and inhibit or abolish PA function.

[0100] Additionally, protein engineering may be employed to improve or alter the characteristics of PA polypeptides. Recombinant DNA technology known to those skilled in the art can be used to create novel mutant proteins or muteins including single or multiple amino acid substitutions, deletions, additions or fusion proteins. Such modified polypeptides can show, e.g., enhanced activity or increased stability. In addition, they may be purified in higher yields and show better solubility than the corresponding natural polypeptide, at least under certain purification and storage conditions. Antibodies of the present invention may bind such modified PA polypeptides.

[0101] Non-naturally occurring variants of PA may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (see e.g., *Carter et al., Nucl. Acids Res. 13*:4331 (1986); and Zoller *et al., Nucl. Acids Res. 10*:6487 (1982)), cassette mutagenesis (see e.g., Wells *et al., Gene 34*:315 (1985)), restriction selection mutagenesis (see e.g., Wells *et al., Philos. Trans. R. Soc. London SerA 317*:415 (1986)).

[0102] Thus, the invention also encompasses antibodies that bind PA derivatives and analogs that have one or more amino acid residues deleted, added, and/or substituted. For

example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges; N-linked glycosylation sites can be altered or eliminated to achieve, for example, expression of a homogeneous product that is more easily recovered and purified from yeast hosts which are known to hyperglycosylate N-linked sites. To this end, a variety of amino acid substitutions at one or both of the first or third amino acid positions on any one or more of the glycosylation recognition sequences in the PA polypeptides and/or an amino acid deletion at the second position of any one or more such recognition sequences will prevent glycosylation of the PA at the modified tripeptide sequence (see, e.g., Miyajimo et al., EMBO J 5(6):1193-1197). Additionally, one or more of the amino acid residues of PA polypeptides (e.g., arginine and lysine residues) may be deleted or substituted with another residue to eliminate undesired processing by proteases such as, for example, furins or kexins.

[0103] The antibodies of the present invention also include antibodies that bind a polypeptide comprising, or alternatively, consisting of a polypeptide comprising, or alternatively, consisting of the polypeptide of SEQ ID NO:2 including the leader; a polypeptide comprising, or alternatively, consisting of the polypeptide of SEQ ID NO:2 minus the amino terminal methionine; a polypeptide comprising, or alternatively, consisting of the polypeptide of SEQ ID NO:2 minus the leader; a polypeptide comprising, or alternatively, consisting of the PA domain I; a polypeptide comprising, or alternatively, consisting of the PA domain II; a polypeptide comprising, or alternatively, consisting of the PA domain III; a polypeptide comprising, or alternatively, consisting of the PA domain IV; a polypeptide comprising, or alternatively, consisting of the PA20 fragment; a polypeptide comprising, or alternatively, consisting of the PA63 fragment; as well as polypeptides which are at least 80% identical, more preferably at least 90% or 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above (the polypeptide and polypeptide fragments of SEQ ID NO:2), and portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

[0104] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a PA polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the PA polypeptide. In other words, to obtain a

polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0105] As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in SEQ ID NO:2 can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

[0106] In a specific embodiment, the identity between a reference (query) sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, is determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter. According to this embodiment, if the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction is made to the results to take into consideration the fact that the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of

the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. A determination of whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of this embodiment. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of this embodiment.

[0107] The present application is also directed to antibodies that bind proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to the PA polypeptide sequence set forth herein as n^1-m^1. In preferred embodiments, the present invention encomapsses antibodies that bind proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific PA N- and C-terminal deletions recited herein.

[0108] In certain preferred embodiments, antibodies of the invention bind PA fusion proteins as described above wherein the PA portion of the fusion protein are those described as n^1-m^1 herein.

Antibodies of the invention may bind Modified PA Polypeptides

[0109] It is specifically contemplated that antibodies of the present invention may bind modified forms of PA proteins SEQ ID NO:2). In specific embodiments, antibodies of the present invention bind PA polypeptides (such as those described above) including, but not limited to naturally purified PA polypeptides, PA polypeptides produced by chemical synthetic procedures, and PA polypeptides produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells using, for example, the recombinant compositions and methods described above. Depending upon the host employed in a recombinant production procedure, the polypeptides may be glycosylated or non-glycosylated. In addition, PA polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

[0110] In addition, antibodies of the present invention may bind PA proteins that were chemically synthesized using techniques known in the art (e.g., see Creighton, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y. (1983), and Hunkapiller, *et al., Nature 310*:105-111 (1984)). For example, a peptide corresponding to a fragment of a PA polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the PA polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0111] The invention additionally encompasses antibodies that bind PA polypeptides that are differentially modified during or after translation, e.g., by glycosylation,

acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin; etc.

[0112] Additional post-translational modifications to PA polypeptides for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[0113] Also provided by the invention are antibodies that bind chemically modified derivatives of PA polypeptides which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0114] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kilodalton and about 100 kilodalton (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500,

13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kilodalton.

[0115] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al., Appl. Biochem. Biotechnol. 56*:59-72 (1996); Vorobjev *et al., Nucleosides Nucleotides 18*:2745-2750 (1999); and Caliceti *et al., Bioconjug. Chem. 10*:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0116] The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, *e.g.*, EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al., Exp. Hematol. 20*:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0117] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0118] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.),

the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (*i.e.*, separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0119] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al., Crit. Rev. Thera. Drug Carrier Sys. 9*:249-304 (1992); Francis *et al., Intern. J. of Hematol. 68*:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0120] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($ClSO_2CH_2CF_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoreothane sulphonyl group.

[0121] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional

polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0122] The number of polyethylene glycol moieties attached to each PA polypeptide (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al., Crit. Rev. Thera. Drug Carrier Sys. 9*:249-304 (1992).

[0123] As mentioned the antibodies of the present invention may bind PA polypeptides that are modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given PA polypeptide. PA polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic PA polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983);

Seifter *et al., Meth Enzymol 182*:626-646 (1990); Rattan *et al., Ann NY Acad Sci 663*:48-62 (1992)).

Anti-PA Antibodies

[0124] In one embodiment, the invention provides antibodies (e.g., antibodies comprising two heavy chains and two light chains linked together by disulfide bridges) that specifically bind PA (SEQ ID NO:2) or fragments or variants thereof, wherein the amino acid sequence of the heavy chain and the amino acid sequence of the light chain are the same as the amino acid sequence of a heavy chain and a light chain of one or more scFvs or cell lines referred to in Table 1. In another embodiment, the invention provides antibodies (each consisting of two heavy chains and two light chains linked together by disulfide bridges to form an antibody) that specifically bind PA or fragments or variants thereof, wherein the amino acid sequence of the heavy chain or the amino acid sequence of the light chain are the same as the amino acid sequence of a heavy chain or a light chain of one or more scFvs or cell lines referred to in Table 1. Immunospecific binding to PA polypeptides may be determined by immunoassays known in the art or described herein for assaying specific antibody-antigen binding. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies that specifically bind to PA are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies molecules, fragments and/or variants (SEQ ID NOS:57-65).

[0125] In one embodiment of the present invention, antibodies that specifically bind to a PA or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of a heavy chain of at least one of the scFvs referred to in Table 1 or cell lines contained in the ATCC Deposits referred to in Table 1 and/or a light chain of at least one of the scFvs referred to in Table 1 or cell lines contained in the ATCC Deposits referred to in Table 1.

[0126] In another embodiment of the present invention, antibodies that specifically bind to PA or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of any one of the VH domains of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1 and/or any one of the VL domains of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In preferred

embodiments, antibodies of the present invention comprise the amino acid sequence of a VH domain and VL domain from a single scFv referred to in Table 1 or single recombinant antibody expressed by a cell line contained in an ATCC Deposit referred to in Table 1. In alternative embodiments, antibodies of the present invention comprise the amino acid sequence of a VH domain and a VL domain from different scFvs referred to in Table 1 or different recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. Molecules comprising, or alternatively consisting of, antibody fragments or variants of the VH and/or VL domains of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1 that specifically bind to PA are also encompassed by the invention, as are nucleic acid molecules encoding these VH and VL domains, molecules, fragments and/or variants (SEQ ID NOS:57-65).

[0127] The present invention also provides antibodies that specifically bind to a polypeptide, or polypeptide fragment or variant of PA, wherein said antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the VH CDRs contained in a VH domain of one or more scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In particular, the invention provides antibodies that specifically bind PA or fragments or variants thereof, comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a VH CDR1 contained in a VH domain of one or more scFvs or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In another embodiment, antibodies that specifically bind PA, comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a VH CDR2 contained in a VH domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In a preferred embodiment, antibodies that specifically bind PA or fragments or variants thereof, comprise, or alternatively consist of a polypeptide having the amino acid sequence of a VH CDR3 contained in a VH domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof, that specifically bind to PA or a PA fragment or variant thereof are also encompassed by the invention, as are nucleic

acid molecules encoding these antibodies, molecules, fragments and/or variants (SEQ ID NOS:57-65).

[0128] The present invention also provides antibodies that specifically bind to a PA polypeptide or a polypeptide fragment or variant of PA, wherein said antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the VL CDRs contained in a VL domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In particular, the invention provides antibodies that specifically bind PA or a fragment or variant thereof, comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a VL CDR1 contained in a VL domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In another embodiment, antibodies that specifically bind PA or a fragment or variant thereof, comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a VL CDR2 contained in a VL domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In a preferred embodiment, antibodies that specifically bind PA or a fragment or variant thereof, comprise, or alternatively consist of a polypeptide having the amino acid sequence of a VL CDR3 contained in a VL domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof, that specifically bind to PA or a PA fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants (SEQ ID NOS:57-65).

[0129] The present invention also provides antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants) that specifically bind to PA polypeptide or a fragment or variant of a PA, wherein said antibodies comprise, or alternatively consist of, one, two, three, or more VH CDRs and one, two, three or more VL CDRs, as contained in a VH domain or VL domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In particular, the invention provides for antibodies that specifically bind to a PA polypeptide or polypeptide fragment

or variant of PA, wherein said antibodies comprise, or alternatively consist of, a VH CDR1 and a VL CDR1, a VH CDR1 and a VL CDR2, a VH CDR1 and a VL CDR3, a VH CDR2 and a VL CDR1, VH CDR2 and VL CDR2, a VH CDR2 and a VL CDR3, a VH CDR3 and a VH CDR1, a VH CDR3 and a VL CDR2, a VH CDR3 and a VL CDR3, or any combination thereof, of the VH CDRs and VL CDRs contained in a VH domain or VL domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In a preferred embodiment, one or more of these combinations are from the same scFv or the same recombinant antibody expressed by cell line contained in an ATCC deposit as disclosed in Table 1. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies, that specifically bind to PA or a fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments or variants (SEQ ID NOS:57-65).

Nucleic Acid Molecules Encoding anti-PA Antibodies

[0130] The present invention also provides for nucleic acid molecules, generally isolated, encoding an antibody of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof).

[0131] In a specific embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a VH domain having an amino acid sequence of any one of the VH domains of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1 and a VL domain having an amino acid sequence of VL domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In another embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a VH domain having an amino acid sequence of any one of the VH domains of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1 or a VL domain having an amino acid sequence of a VL domain of at least one of the

scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1.

[0132] The present invention also provides antibodies that comprise, or alternatively consist of, variants (including derivatives) of the antibody molecules (e.g., the VH domains and/or VL domains) described herein, which antibodies specifically bind to PA or a fragment or variant thereof. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH domain, VHCDR1, VHCDR2, VHCDR3, VL domain, VLCDR1, VLCDR2, or VLCDR3. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (e.g., the ability to bind PA).

[0133] For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations may be silent or neutral missense mutations, i.e., have no, or little, effect on an antibody's ability to bind antigen. These types of mutations may be useful to optimize codon usage, or improve a hybridoma's antibody production. Alternatively, non-neutral missense mutations may alter an antibody's ability to bind antigen. The location of most silent and neutral missense

mutations is likely to be in the framework regions, while the location of most non-neutral missense mutations is likely to be in CDR, though this is not an absolute requirement. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (e.g, improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein may routinely be expressed and the functional and/or biological activity of the encoded protein, (e.g., ability to specifically bind PA) can be determined using techniques described herein or by routinely modifying techniques known in the art.

[0134] In a specific embodiment, an antibody of the invention (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that specifically binds PA or a fragment or variant thereof, comprises, or alternatively consists of, an amino acid sequence encoded by a nucleotide sequence that hybridizes to a nucleotide sequence that is complementary to that encoding one of the VH or VL domains of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1 under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45° C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65° C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45° C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68° C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3). The nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0135] It is well known within the art that polypeptides, or fragments or variants thereof, with similar amino acid sequences often have similar structure and many of the same biological activities. Thus, in one embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that specifically binds to PA or fragments or variants of PA, comprises, or alternatively consists of, a VH domain having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least

75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to the amino acid sequence of a VH domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1.

[0136] In another embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that specifically binds to PA or a fragment or variant of PA, comprises, or alternatively consists of, a VL domain having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to the amino acid sequence of a VL domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1.

Methods of Producing Antibodies

[0137] Antibodies in accordance with the invention were prepared via the utilization of a phage scFv display library. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed herein.

[0138] In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of lymphoid tissues) or synthetic cDNA libraries. The DNA encoding the VH and VL domains are joined together by an scFv linker by PCR and cloned into a phagemid vector (*e.g.*, pCANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to an antigen of interest (*i.e.*, a PA polypeptide or a fragment thereof) can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include, but are not limited to, those disclosed in Brinkman *et al.*, J. Immunol. Methods 182:41-50 (1995); Ames *et al.*, J. Immunol. Methods 184:177-186 (1995);

Kettleborough *et al.*, Eur. J. Immunol. 24:952-958 (1994); Persic *et al.*, Gene 187 9-18 (1997); Burton *et al.*, Advances in Immunology 57:191-280(1994); PCT application No. PCT/GB91/O1 134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18719; WO 93/1 1236; WO 95/15982; WO 95/20401; WO97/13844; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,717; 5,780,225; 5,658,727; 5,735,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0139] For some uses, such as for in vitro affinity maturation of an antibody of the invention, it may be useful to express the VH and VL domains of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1 as single chain antibodies or Fab fragments in a phage display library. For example, the cDNAs encoding the VH and VL domains of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1 may be expressed in all possible combinations using a phage display library, allowing for the selection of VH/VL combinations that bind PA polypeptides with preferred binding characteristics such as improved affinity or improved off rates. Additionally, VH and VL segments— and in particular, the CDR regions of the VH and VL domains of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, in particular, may be mutated in vitro. Expression of VH and VL domains with "mutant" CDRs in a phage display library allows for the selection of VH/VL combinations that bind PA polypeptides with preferred binding characteristics such as improved affinity or improved off rates.

[0140] In particular embodiments, antibodies of the invention comprise the VH and VL domains of the PWD0587 scFv wherein the VH domain contains one or more of the following mutations (using amino acid numbering according to that of SEQ ID NO:53): Q13R, S31W, I100V, and/or E105D. An antibody comprising the PWD0587 VH domain with the Q13R, S31W and I100V mutations and the PWD0587 VL domain, had an approximately 11 fold increase in affinity for the PA antigen compared to an antibody comprising the PWD0587 heavy and light chains. Thus, in specific embodiments, an antibody of the invention comprises the PWD0587 VH domain with the Q13R, S31W and I100V mutations and the PWD0587 VL domain.

[0141] An antibody comprising the PWD0587 VH domain with the Q13R and S31W mutations and the PWD0587 VL domain, had an approximately 68 fold increase in affinity for the PA antigen compared to an antibody comprising the PWD0587 heavy and light chains. Thus, in specific embodiments, an antibody of the invention comprises the PWD0587 VH domain with the Q13R and S31W mutations and the PWD0587 VL domain.

[0142] An antibody comprising the PWD0587 VH domain with the Q13R, S31W, I100V and E105D mutations and the PWD0587 VL domain, had an approximately 121 fold increase in affinity for the PA antigen compared to an antibody comprising the PWD0587 heavy and light chains. Thus, in specific embodiments, an antibody of the invention comprises the PWD0587 VH domain with the Q13R, S31W, I100V and E105D mutations and the PWD0587 VL domain.

[0143] An antibody comprising the PWD0587 VH domain with the Q13R, S31W and E105D mutations and the PWD0587 VL domain, had an approximately 665 fold increase in affinity for the PA antigen compared to an antibody comprising the PWD0587 heavy and light chains. Thus in specific embodiment an antibody of the invention comprises the PWD0587 VH domain with the Q13R, S31W and E105D mutations and the PWD0587 VL domain.

[0144] Preliminary testing of the four mutant forms of the PWD0587 antibody with increased affinities for PA compared to the parental PWD0587 (unmutated) antibody, indicated that the mutant PWD0587 antibodies behaved comparably to the parental PWD0587 antibody in, for example, a rubidium release assay (e.g., similar to the assays described in Example 5). In a rat lethal toxin challenge model (similar to the assays described in Example 9) an antibody comprising the PWD0587 VH domain with the Q13R, S31W and E105D mutations and the PWD0587 VL domain was slightly more effective than the parental PWD0587 antibody in preventing lethal toxin induced death.

Additional Methods of Producing Antibodies

[0145] Antibodies of the invention (including antibody fragments or variants) can be produced by any method known in the art. For example, it will be appreciated that antibodies in accordance with the present invention can be expressed in cell lines including, but not limited to, myeloma cell lines and hybridoma cell lines. Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for

transformation of a suitable mammalian or nonmammalian host cells or to generate phage display libraries, for example. Additionally, polypeptide antibodies of the invention may be chemically synthesized or produced through the use of recombinant expression systems.

[0146] One way to produce the antibodies of the invention would be to clone the VH and/or VL domains of an scFv referred to in Table 1 or recombinant antibody expressed by the cell lines contained in the ATCC Deposits referred to in Table 1. In order to isolate the VH and VL domains from bacteria transfected with a vector containing the scFv, PCR primers complementary to VH or VL nucleotide sequences (See Example 6), may be used to amplify the VH and VL sequences. The PCR products may then be cloned using vectors, for example, which have a PCR product cloning site consisting of a 5' and 3' single T nucleotide overhang, that is complementary to the overhanging single adenine nucleotide added onto the 5' and 3' end of PCR products by many DNA polymerases used for PCR reactions. The VH and VL domains can then be sequenced using conventional methods known in the art. Alternatively, the VH and VL domains may be amplified using vector specific primers designed to amplify the entire scFv, (i.e. the VH domain, linker and VL domain.)

[0147] The cloned VH and VL genes may be placed into one or more suitable expression vectors. By way of non-limiting example, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site may be used to amplify the VH or VL sequences. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains may be cloned into vectors expressing the appropriate immunoglobulin constant region, *e.g.*, the human IgG1 or IgG4 constant region for VH domains, and the human kappa or lambda constant regions for kappa and lambda VL domains, respectively. Preferably, the vectors for expressing the VH or VL domains comprise a promoter suitable to direct expression of the heavy and light chains in the chosen expression system, a secretion signal, a cloning site for the immunoglobulin variable domain, immunoglobulin constant domains, and a selection marker such as neomycin. The VH and VL domains may also be cloned into a single vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art (See, for example, Guo et al., J. Clin. Endocrinol. Metab. 82:925-

31 (1997), and Ames et al., J. Immunol. Methods 184:177-86 (1995) which are herein incorporated in their entireties by reference).

[0148] The invention provides polynucleotides comprising, or alternatively consisting of, a nucleotide sequence encoding an antibody of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof). The invention also encompasses polynucleotides that hybridize under high stringency, or alternatively, under intermediate or lower stringency hybridization conditions, *e.g.*, as defined *supra*, to polynucleotides complementary to nucleic acids having a polynucleotide sequence that encodes an antibody of the invention or a fragment or variant thereof.

[0149] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. If the amino acid sequences of the VH domains, VL domains and CDRs thereof, are known, nucleotide sequences encoding these antibodies can be determined using methods well known in the art, *i.e.*, the nucleotide codons known to encode the particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody, of the invention. Such a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0150] Alternatively, a polynucleotide encoding an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells or Epstein Barr virus transformed B cell lines that express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated

by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0151] Once the nucleotide sequence of the antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0152] In a specific embodiment, VH and VL domains of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or fragments or variants thereof, are inserted within framework regions using recombinant DNA techniques known in the art. In a specific embodiment, one, two, three, four, five, six, or more of the CDRs of a VH and/or a VL domain of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or fragments or variants thereof, are inserted within framework regions using recombinant DNA techniques known in the art. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, *e.g.*, Chothia *et al.*, J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions, the contents of which are hereby incorporated by reference in its entirety). Preferably, the polynucleotides generated by the combination of the framework regions and CDRs encode an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that specifically binds to a PA polypeptide. Preferably, as discussed *supra*, polynucleotides encoding variants of antibodies or antibody fragments having one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions do not significantly alter binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region

cysteine residues participating in an intrachain disulfide bond to generate antibody molecules, or antibody fragments or variants, lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and fall within the ordinary skill of the art.

[0153] The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

[0154] An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (mAbs) an important milestone towards fulfilling the promise of antibody therapy in human disease.

[0155] Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized Monoclonal antibodies and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as cancer, which require repeated antibody administrations.

[0156] One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including

human antigens. Using the hybridoma technology, antigen-specific human Monoclonal antibodies with the desired specificity could be readily produced and selected.

[0157] This general strategy was demonstrated in connection with the generation of the first XenoMouse™ strains as published in 1994. *See* Green et al. *Nature Genetics* 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes (YACS) containing germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. Id. The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human monoclonal antibodies. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce XenoMouse™ mice. *See* Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J Exp. Med.* 188:483-495 (1998), Green, *Journal of Immunological Methods* 231:11-23 (1999) and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by reference.

[0158] Such approach is further discussed and delineated in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/710,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30, 1992, filed 08/031,801, filed March 15,1993, 08/112,848, filed August 27, 1993, 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, April 27, 1995, 0-8/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/471,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 08/486,853, filed June 5, 1995, 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, and 08/759,620, filed December 3, 1996. *See also* Mendez et al. *Nature Genetics* 15:146-156 (1997) and Green and Jakobovits J *Exp. Med.* 188:483 495 (1998). *See also* European

Patent No., EP 0 463 151 B1, grant published June 12, 1996, International Patent Application No., WO 94/02602, published February 3, 1994, International Patent Application No., WO 96/34096, published October 31, 1996, and WO 98/24893, published June 11, 1998. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

[0159] Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a murine variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody. Thus, it would be desirable to provide fully human antibodies against PA polypeptides in order to vitiate concerns and/or effects of HAMA or HACA responses.

[0160] Monoclonal antibodies specific for PA polypeptides may be prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 571-681 (1981)). Briefly, XenoMouse™ mice may be immunized with PA polypeptides. After immunization, the splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line, such as the myeloma cell line (SP2O), available from the ATCC, may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the PA polypeptides.

[0161] For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use human or chimeric antibodies. Completely human antibodies are particularly desirable for therapeutic treatment of human patients. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50435, WO 98/24893, WO98/16654, WO 96/34096, WO 96/35735, and WO 91/10741; each of which is incorporated herein by reference in its entirety. In a specific embodiment, antibodies of the present invention comprise one or more VH and VL

domains of the invention and constant regions from another immunoglobulin molecule, preferably a human immunoglobulin molecule. In a specific embodiment, antibodies of the present invention comprise one or more CDRs corresponding to the VH and VL domains of the invention and framework regions from another immunoglobulin molecule, preferably a human immunoglobulin molecule. In other embodiments, an antibody of the present invention comprises one, two, three, four, five, six or more VL CDRs or VH CDRs corresponding to one or more of the VH or VL domains of one or more scFvs referred to in Table 1 or one or more recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or fragments or variants thereof, and framework regions (and, optionally one or more CDRs not present in the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1) from a human immunoglobulin molecule. In a preferred embodiment, an antibody of the present invention comprises a VH CDR3, VL CDR3, or both, corresponding to the same recombinant antibody, or different recombinant antibodies selected from the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or fragments or variants thereof, and framework regions from a human immunoglobulin.

[0162] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a human variable region and a non-human (e.g., murine) immunoglobulin constant region or vice versa. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, Science 229:1202 (1985); Oi *et al.*, BioTechniques 4:214 (1986); Gillies *et al.*, J. Immunol. Methods 125:191-202 (1989); U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Chimeric antibodies comprising one or more CDRs from human species and framework regions from a non-human immunoglobulin molecule (*e.g.*, framework regions from a murine, canine or feline immunoglobulin molecule) (or vice versa) can be produced using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka *et al.*, Protein Engineering 7(6):805-814 (1994); Roguska *et al.*, PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,352). In a preferred embodiment, chimeric antibodies comprise a human CDR3

having an amino acid sequence of any one of the VH CDR3s or VL CDR3s of a VH or VL domain of one or more of the scFvs referred to in Table 1, or a variant thereof, and non-human framework regions or human framework regions different from those of the frameworks in the corresponding scFv disclosed in Table 1. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; Riechmann *et al.*, Nature 352:323 (1988), which are incorporated herein by reference in their entireties.)

[0163] Intrabodies are antibodies, often scFvs, that are expressed from a recombinant nucleic acid molecule and engineered to be retained intracellularly (e.g., retained in the cytoplasm, endoplasmic reticulum, or periplasm). Intrabodies may be used, for example, to ablate the function of a protein to which the intrabody binds. The expression of intrabodies may also be regulated through the use of inducible promoters in the nucleic acid expression vector comprising the intrabody. Intrabodies of the invention can be produced using methods known in the art, such as those disclosed and reviewed in Chen *et al., Hum. Gene Ther. 5*:595-601 (1994); Marasco, W.A., *Gene Ther. 4*:11-15 (1997); Rondon and Marasco, *Annu. Rev. Microbiol. 51*:257-283 (1997); Proba *et al., J. Mol. Biol. 275*:245-253 (1998); Cohen *et al., Oncogene 17*:2445-2456 (1998); Ohage and Steipe, *J. Mol. Biol. 291*:1119-1128 (1999); Ohage *et al., J. Mol. Biol. 291*:1129-1134 (1999); Wirtz and Steipe, *Protein Sci. 8*:2245-2250 (1999); Zhu *et al., J. Immunol. Methods 231*:207-222 (1999); and references cited therein.

[0164] Recombinant expression of an antibody of the invention (including antibody fragments or variants thereof (*e.g.*, a heavy or light chain of an antibody of the invention), requires construction of an expression vector(s) containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule (e.g., a whole antibody, a heavy or light chain of an antibody, or portion thereof (preferably, but not necessarily, containing the heavy or light chain variable domain)), of the invention has been obtained, the vector(s) for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding

nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention (e.g., a whole antibody, a heavy or light chain of an antibody, a heavy or light chain variable domain of an antibody, or a portion thereof, or a heavy or light chain CDR, a single chain Fv, or fragments or variants thereof), operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464, the contents of each of which are hereby incorporated by reference in its entirety) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy chain, the entire light chain, or both the entire heavy and light chains.

[0165] The expression vector(s) is(are) transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing polynucleotide(s) encoding an antibody of the invention (e.g., whole antibody, a heavy or light chain thereof, or portion thereof, or a single chain antibody, or a fragment or variant thereof), operably linked to a heterologous promoter. In preferred embodiments, for the expression of entire antibody molecules, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0166] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include, but are not limited to, bacteriophage particles engineered to express antibody fragments or variants thereof (single chain antibodies), microorganisms such as bacteria (*e.g., E. coli, B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast

(*e.g.*, *Saccharomyces, Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3, NS0 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, Gene 45:101 (1986); Cockett *et al.*, Bio/Technology 8:2 (1990); Bebbington et al., Bio/Techniques 10:169 (1992); Keen and Hale, Cytotechnology 18:207 (1996)). These references are incorporated in their entireties by reference herein.

[0167] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, EMBO 1. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione 5-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include

thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0168] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) may be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. Antibody coding sequences may be cloned individually into non-essential regions (for example, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedrin promoter).

[0169] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region El or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, Proc. Natl. Acad. Sci. USA 8 1:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bittner *et al.*, Methods in Enzymol. 153:51-544 (1987)).

[0170] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO,

VERY, BHK, HeLa, COS, NSO, MDCK, 293, 3T3, W138, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT2O and T47D, and normal mammary gland cell line such as, for example, CRL7O3O and HsS78Bst.

[0171] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

[0172] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy *et al.*, Cell 22:8 17 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler *et al.*, Natl. Acad. Sci. USA 77:357 (1980); O'Hare *et al.*, Proc. Natl. Acad. Sci. USA 78:1527 (1981)); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 (Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62: 191-217 (1993); TIB TECH 11(5):155-2 15 (May, 1993)); and *hygro*, which confers resistance to hygromycin (Santerre *et al.*, Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for

example, in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin *et al.*, J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[0173] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, "The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells" in *DNA Cloning, Vol.3.* (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the coding sequence of the antibody, production of the antibody will also increase (Crouse *et al.*, Mol. Cell. Biol. 3:257 (1983)).

[0174] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers, including, for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are incorporated in their entireties by reference herein.

[0175] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides.

Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain is preferably placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2 197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0176] Once an antibody molecule of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) has been chemically synthesized or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, or more generally, a protein molecule, such as, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies of the present invention may be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

[0177] Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the antibodies of the present invention may be glycosylated or may be non-glycosylated. In addition, antibodies of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

[0178] Antibodies of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller, M., et al., 1984, Nature 310:105-111). For example, a peptide corresponding to a fragment of an antibody of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the antibody polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine,

norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[0179] The invention encompasses antibodies which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

[0180] Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The antibodies may also be modified with a detectable label, such as an enzymatic, fluorescent, radioisotopic or affinity label to allow for detection and isolation of the antibody.

[0181] Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, glucose oxidase or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include biotin, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi, or other radioisotopes such as, for example, iodine (131I, 125I, 123I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (115mIn, 113mIn, 112In, 111In), and technetium (99Tc, 99mTc), thallium (201Ti), gallium (68Ga, 67Ga), palladium (103Pd), molybdenum (99Mo), xenon (133Xe), fluorine (18F), 153Sm, 177Lu, 159Gd,

149Pm, 140La, 175Yb, 166Ho, 90Y, 47Sc, 186Re, 188Re, 142Pr, 105Rh, 97Ru, 68Ge, 57Co, 65Zn, 85Sr, 32P, 153Gd, 169Yb, 51Cr, 54Mn, 75Se, 113Sn, and 117Tin.

[0182] In specific embodiments, antibodies of the invention may be labeled with Europium. For example, antibodies of the invention may be labelled with Europium using the DELFIA Eu-labeling kit (catalog# 1244-302, Perkin Elmer Life Sciences, Boston, MA) following manufacturer's instructions.

[0183] In specific embodiments, antibodies of the invention are attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, 111In, 177Lu, 90Y, 166Ho, 153Sm, 215Bi and 225Ac to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators attached to antibodies of the invention is 111In. In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to antibodies polypeptides of the invention is 90Y. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA). In specific embodiments, the macrocyclic chelator is □-(5-isothiocyanato-2-methoxyphenyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetraacetic acid. In other specific embodiments, the DOTA is attached to the antibody of the invention via a linker molecule. Examples of linker molecules useful for conjugatinga macrocyclic chelator such as DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90, 1998; Peterson et al., Bioconjug. Chem. 10(4):553-7, 1999; and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entireties.

[0184] In one embodiment, antibodies of the invention are labeled with biotin. In other related embodiments, biotinylated antibodies of the invention may be used, for example, as an imaging agent or as a means of identifying one or more TRAIL receptor coreceptor or ligand molecules.

[0185] Also provided by the invention are chemically modified derivatives of antibodies of the invention which may provide additional advantages such as increased solubility, stability and in vivo or in vitro circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol,

ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The antibodies may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

[0186] The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[0187] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo et al., Appl. Biochem. Biotechnol. 56:59-72 (1996); Vorobjev et al., Nucleosides Nucleotides 18:2745-2750 (1999); and Caliceti et al., Bioconjug. Chem. 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

[0188] The polyethylene glycol molecules (or other chemical moieties) should be attached to the antibody with consideration of effects on functional or antigenic domains of the antibody. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include, for example, lysine residues and the N-terminal amino acid residues; those having a free carboxyl group

may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[0189] As suggested above, polyethylene glycol may be attached to proteins, e.g., antibodies, via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[0190] One may specifically desire antibodies chemically modified at the N-terminus of either the heavy chain or the light chain or both. Using polyethylene glycol as an illustration, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective chemical modification at the N-terminus may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[0191] As indicated above, pegylation of the antibodies of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the antibody either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992); Francis et al., Intern. J. of Hematol. 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[0192] One system for attaching polyethylene glycol directly to amino acid residues of antibodies without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride (ClSO2CH2CF3). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes antibody-polyethylene glycol conjugates produced by reacting antibodies of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoreothane sulphonyl group.

[0193] Polyethylene glycol can also be attached to antibodies using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Antibody-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the antibody by a linker can also be produced by reaction of antibodies with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated antibody products produced using the reaction chemistries set out herein are included within the scope of the invention.

[0194] The number of polyethylene glycol moieties attached to each antibody of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated antibodies of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per antibody molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992).

Characterization of anti-PA Antibodies

[0195] Antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) may also be described or specified in terms of their binding to PA polypeptides or fragments or variants of PA

polypeptides. In specific embodiments, antibodies of the invention bind PA polypeptides, or fragments or variants thereof, with a dissociation constant or K_D of less than or equal to 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, or 10^{-5} M. More preferably, antibodies of the invention bind PA polypeptides or fragments or variants thereof with a dissociation constant or K_D less than or equal to 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, or 10^{-8} M. Even more preferably, antibodies of the invention bind PA polypeptides or fragments or variants thereof with a dissociation constant or K_D less than or equal to 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M. The invention encompasses antibodies that bind PA polypeptides with a dissociation constant or K_D that is within any one of the ranges that are between each of the individual recited values.

[0196] In specific embodiments, antibodies of the invention bind PA polypeptides or fragments or variants thereof with an off rate (k_{off}) of less than or equal to 5×10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5×10^{-3} sec^{-1} or 10^{-3} sec^{-1}. More preferably, antibodies of the invention bind PA polypeptides or fragments or variants thereof with an off rate (k_{off}) less than or equal to 5×10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5×10^{-5} sec^{-1}, or 10^{-5} sec^{-1} 5×10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5×10^{-7} sec^{-1} or 10^{-7} sec^{-1}. The invention encompasses antibodies that bind PA polypeptides with an off rate (k_{off}) that is within any one of the ranges that are between each of the individual recited values.

[0197] In other embodiments, antibodies of the invention bind PA polypeptides or fragments or variants thereof with an on rate (k_{on}) of greater than or equal to 10^3 M^{-1} sec^{-1}, 5×10^3 M^{-1} sec^{-1}, 10^4 M^{-1} sec^{-1} or 5×10^4 M^{-1} sec^{-1}. More preferably, antibodies of the invention bind PA polypeptides or fragments or variants thereof with an on rate (k_{on}) greater than or equal to 10^5 M^{-1} sec^{-1}, 5×10^5 M^{-1} sec^{-1}, 10^6 M^{-1} sec^{-1}, or 5×10^6 M^{-1} sec^{-1} or 10^7 M^{-1} sec^{-1}. The invention encompasses antibodies that bind PA polypeptides with on rate (k_{on}) that is within any one of the ranges that are between each of the individual recited values.

[0198] In preferred embodiments, the antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), specifically bind to PA polypeptides and do not cross-react with any other antigens. In preferred embodiments, the antibodies of the invention specifically bind to PA polypeptides (e.g., SEQ ID NO:2 or fragments or variants thereof) and do not cross-

react with other bacterial binary toxins (A-B toxins) such as those from *Clostridum difficile, Clostridium perfringens, Clostridium spiroforme, Clostridium botulinum, Bacillus cereus and/or Bacillus thuringiensis.*

[0199] In another embodiment, the antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), specifically bind to PA polypeptides and cross-react with other antigens. In other embodiments, the antibodies of the invention specifically bind to PA polypeptides (e.g., SEQ ID NO:2 or fragments or variants thereof) and cross-react with other bacterial binary toxins (A-B toxins) such as those from *Clostridum difficile, Clostridium perfringens, Clostridium spiroforme, Clostridium botulinum, Bacillus cereus and/or Bacillus thuringiensis.*

[0200] In a preferred embodiment, antibodies of the invention preferentially bind PA (SEQ ID NO:2), or fragments and variants thereof relative to their ability to bind other antigens (e.g., other bacterial binary toxins (A-B toxins) such as those from *Clostridum difficile, Clostridium perfringens, Clostridium spiroforme, Clostridium botulinum, Bacillus cereus and/or Bacillus thuringiensis*). An antibody's ability to preferentially bind one antigen compared to another antigen may be determined using any method known in the art.

[0201] By way of non-limiting example, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with a dissociation constant (K_D) that is less than the antibody's K_D for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity (i.e., K_D) that is at least one order of magnitude less than the antibody's K_D for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity (i.e., K_D) that is at least two orders of magnitude less than the antibody's K_D for the second antigen.

[0202] In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an off rate (k_{off}) that is less than the antibody's k_{off} for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with a k_{off} that is at least one order of magnitude less than the antibody's k_{off} for the second antigen. In another non-limiting embodiment, an antibody may be considered

to bind a first antigen preferentially if it binds said first antigen with a k_{off} that is at least two orders of magnitude less than the antibody's k_{off} for the second antigen.

[0203] The invention also encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that have one or more of the same biological characteristics as one or more of the antibodies described herein. By "biological characteristics" is meant, the in vitro or in vivo activities or properties of the antibodies, such as, for example, the ability to bind to PA polypeptides (e.g., either the PA83 or PA63 form of PA); or the ability to inhibit the cleavage of the PA83 into PA20 and PA63 by proteases such as trypsin or furin. Additionally, antibodies of the invention may: prevent oligomerization of PA63, especially heptamerization of PA63; inhibit or abolish the ability of PA63 to bind to an anthrax receptor, e.g., ATR and/or CMG2 (See Example 3); inhibit or abolish the ability of PA63 to bind LF or EF; inhibit or abolish the ability of PA63 to form pores in membranes (see Example 5); inhibit or abolish the ability of lethal toxin (LT) to kill cells, such as macrophages (see Example 8), or animals (see Examples 9-12); or inhibit or abolish the ability of PA heptamers to translocate LF or EF across a membrane (see Example 13). Optionally, the antibodies of the invention will bind to the same epitope as at least one of the antibodies specifically referred to herein. Such epitope binding can be routinely determined using assays known in the art.

[0204] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that inhibit or abolish biological activities of PA. By "biological activities of PA" is meant, for example, the ability of PA83 to be cleaved by proteases into PA20 and PA63 fragments; the ability of PA to bind to ATR and/or CMG2; the ability of PA or PA63 to oligomerize, especially to heptamerize; the ability of PA63 to bind LF or EF; the ability of PA63 heptamers to form pores in a membrane; and/or the ability of PA heptamers to translocate EF or LF across a membrane. In one embodiment, an antibody that inhibits or abolishes biological activities of PA comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a fragment or variant thereof. In a specific embodiment, an antibody that inhibits or abolishes biological activities of PA comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in

Table 1, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0205] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that inhibit the cleavage of the PA83 into PA20 and PA63 by proteases such as trypsin or furin. See, e.g., Example 2 wherein an antibody that binds peptides that span the RKKR cleavage site of PA may be predictive of an antibody's ability to inhibit the cleavage of PA by proteases. Alternatively, a PA cleavage assay is described in *J. Biol. Chem.* (1992), 267:16396-402, which is hereby incorporated by reference in its entirety. In one embodiment, an antibody that inhibits the cleavage of the PA83 into PA20 and PA63 comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a fragment or variant thereof. In a specific embodiment, an antibody that inhibits the cleavage of the PA83 into PA20 and PA63 comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0206] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that block or inhibit the binding of PA to ATR and/or CMG2 (e.g., see Example 3). In one embodiment, an antibody that blocks or inhibits the binding of PA to ATR and/or CMG2 comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. In a specific embodiment, an antibody that blocks or inhibits the binding of PA to ATR and/or CMG2 comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1 or any one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0207] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that

block or inhibit the ability of PA or PA63 to heptamerize. In one embodiment, an antibody that blocks or inhibits the ability of PA or PA63 to heptamerize comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. In a specific embodiment, an antibody that blocks or inhibits the ability of PA63 to heptamerize comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1 or any one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0208] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that block or inhibit the ability of PA63 to bind EF or LF. In one embodiment, an antibody that blocks or inhibits the ability of PA63 to bind EF or LF comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. In a specific embodiment, an antibody that blocks or inhibits the ability of PA63 to bind EF or LF comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1 or any one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0209] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that block or inhibit the ability of PA63 heptamers to form pores in membranes. In one embodiment, an antibody that blocks or inhibits the ability of PA63 heptamers to form pores in membranes comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. In a specific embodiment, an antibody that blocks or inhibits the ability of PA63 heptamers to form pores in membranes comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1 or any

one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0210] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that block or inhibit the ability of PA63 heptamers to translocate EF or LF across membranes. In one embodiment, an antibody that blocks or inhibits the ability of PA63 heptamers to translocate EF or LF across membranes comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. In a specific embodiment, an antibody that blocks or inhibits the ability of PA63 heptamers to translocate EF or LF across membranes comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1 or any one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0211] The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that block or inhibit the ability of anthrax lethal toxin to kill cells or animals. In one embodiment, an antibody that blocks or inhibits the ability of anthrax lethal toxin to kill cells or animals comprises, or alternatively consists of a VH and/or a VL domain of at least one of the scFvs referred to in Table 1 or at least one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. In a specific embodiment, an antibody that blocks or inhibits the ability of anthrax lethal toxin to kill cells or animals comprises, or alternatively consists of a VH and a VL domain of any one of the scFvs referred to in Table 1 or any one of the recombinant antibodies expressed by the cell lines contained in the ATCC Deposits referred to in Table 1, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

[0212] The present invention also provides for fusion proteins comprising, or alternatively consisting of, an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that specifically bind to PA fused to

a heterologous polypeptide. Preferably, the heterologous polypeptide to which the antibody is fused is useful for function or is useful to target the fusion protein to cells with surface bound PA molecules. In specific embodiments the invention encompasses bispecific antibodies in which one antibody binding site is specific for PA and the second antibody binding site is specific for a heterologous polypeptide. In one embodiment, a fusion protein of the invention comprises, or alternatively consists of, a polypeptide having the amino acid sequence of any one or more of the VH domains of an antibody of the invention or the amino acid sequence of any one or more of the VL domains of an antibody of the invention or fragments or variants thereof, and a heterologous polypeptide sequence. In another embodiment, a fusion protein of the present invention comprises, or alternatively consists of, a polypeptide having the amino acid sequence of any one, two, three, or more of the VH CDRs of an antibody of the invention, or the amino acid sequence of any one, two, three, or more of the VL CDRs of an antibody of the invention, or fragments or variants thereof, and a heterologous polypeptide sequence. In a preferred embodiment, the fusion protein comprises, or alternatively consists of, a polypeptide having the amino acid sequence of, a VH CDR3 of an antibody of the invention, or fragment or variant thereof, and a heterologous polypeptide sequence, which fusion protein specifically binds to PA. In another embodiment, a fusion protein comprises, or alternatively consists of a polypeptide having the amino acid sequence of at least one VH domain of an antibody of the invention and the amino acid sequence of at least one VL domain of an antibody of the invention or fragments or variants thereof, and a heterologous polypeptide sequence. Preferably, the VH and VL domains of the fusion protein correspond to a single antibody (or scFv or Fab fragment) of the invention. In yet another embodiment, a fusion protein of the invention comprises, or alternatively consists of a polypeptide having the amino acid sequence of any one, two, three or more of the VH CDRs of an antibody of the invention and the amino acid sequence of any one, two, three or more of the VL CDRs of an antibody of the invention, or fragments or variants thereof, and a heterologous polypeptide sequence. Preferably, two, three, four, five, six, or more of the VHCDR(s) or VLCDR(s) correspond to single antibody (or scFv or Fab fragment) of the invention. Nucleic acid molecules encoding these fusion proteins are also encompassed by the invention.

[0213] Antibodies of the present invention (including antibody fragments or variants thereof) may be characterized in a variety of ways. In particular, antibodies and related

molecules of the invention may be assayed for the ability to specifically bind to PA or a fragment or variant of PA, using techniques described herein or routinely modifying techniques known in the art. Assays for the ability of the antibodies of the invention to specifically bind PA or a fragment or variant of PA, may be performed in solution (*e.g.*, Houghten, Bio/Techniques 13:412-421(1992)), on beads (*e.g.*, Lam, Nature 354:82-84 (1991)), on chips (*e.g.*, Fodor, Nature 364:555-556 (1993)), on bacteria (*e.g.*, U.S. Patent No. 5,223,409), on spores (*e.g.*, Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (*e.g.*, Cull et al., Proc. Natl. Acad. Sci. USA 89:1865-1869 (1992)) or on phage (*e.g.*, Scott and Smith, Science 249:386-390 (1990); Devlin, Science 249:404-406 (1990); Cwirla et al., Proc. Natl. Acad. Sci. USA 87:7178-7182 (1990); and Felici, J. Mol. Biol. 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). Antibodies that have been identified to specifically bind to PA or a fragment or variant of PA can then be assayed for their specificity and affinity for PA using or routinely modifying techniques described herein or otherwise known in the art (see, e.g., Examples 1 and 2).

[0214] The antibodies of the invention may be assayed for specific binding to PA polypeptides and cross-reactivity with other antigens by any method known in the art. Immunoassays which can be used to analyze specific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as BIAcore analysis, FACS (fluorescence activated cell sorter) analysis, immunofluorescence, immunocytochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, western blots, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

[0215] ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a

period of time, washing away unbound antibodies or non-specifically bound antibodies, and detecting the presence of the antibodies specifically bound to the antigen coating the well. In ELISAs, the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Alternatively, the antigen need not be directly coated to the well; instead the ELISA plates may be coated with an anti-Ig Fc antibody, and the antigen in the form or a PA-Fc fusion protein, may be bound to the anti-Ig Fc coated to the plate. This may be desirable so as to maintain the antigen protein (e.g., the PA polypeptides) in a more native conformation than it may have when it is directly coated to a plate. In another alternative, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

[0216] The binding affinity of an antibody (including an scFv or other molecule comprising, or alternatively consisting of, antibody fragments or variants thereof) to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*, antigen labeled with ^{3}H or ^{125}I), or fragment or variant thereof with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of the present invention for PA and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, a PA polypeptide is incubated with an antibody of the present invention conjugated to a labeled compound (*e.g.*, compound labeled with ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second anti-PA antibody. Assays for determining the ability of one antibody to competitively inhibit the binding of another antibody are known in the art (See, for example, Harlow, Ed & David Lane, *Antibodies: A Laboratory Manual*, New York: Cold Spring Harbor Laboratory, 1988. pp. 567-569.) This kind of competitive

assay between two antibodies, may also be used to determine if two antibodies bind the same, closely associated (e.g., overlapping) or different epitopes.

[0217] In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibodies (including antibody fragments or variants thereof) to PA, or fragments of PA.

[0218] Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40 degrees C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40 degrees C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

[0219] Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, *e.g.*, an anti-human antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*, ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the

background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al., eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

Antibody Conjugates

[0220] The present invention encompasses antibodies (including antibody fragments or variants thereof), recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a heterologous polypeptide (or portion thereof, preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids of the polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. For example, antibodies of the invention may be used to target heterologous polypeptides to particular cell types (*e.g.*, cancer cells), either *in vitro* or *in vivo*, by fusing or conjugating the heterologous polypeptides to antibodies of the invention that are specific for particular cell surface antigens or which bind antigens that bind particular cell surface receptors. Antibodies of the invention may also be fused to albumin (including but not limited to recombinant human serum albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide). Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention. Such fusion proteins may, for example, facilitate purification and may increase half-life in vivo. Antibodies fused or conjugated

to heterologous polypeptides may also be used in *in vitro* immunoassays and purification methods using methods known in the art. See *e.g.*, Harbor *et al.*, supra, and PCT publication WO 93/2 1232; EP 439,095; Naramura *et al.*, Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies *et al.*, PNAS 89:1428-1432 (1992); Fell *et al.*, J. Immunol. 146:2446-2452 (1991), which are incorporated by reference in their entireties.

[0221] The present invention further includes compositions comprising, or alternatively consisting of, heterologous polypeptides fused or conjugated to antibody fragments. For example, the heterologous polypeptides may be fused or conjugated to a Fab fragment, Fd fragment, Fv fragment, $F(ab)_2$ fragment, or a portion thereof. Methods for fusing or conjugating polypeptides to antibody portions are known in the art. See, *e.g.*, U.S. Patent Nos. 5,356,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 9 1/06570; Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA 88: 10535-10539 (1991); Zheng *et al.*, J. Immunol. 154:5590-5600 (1995); and Vil *et al.*, Proc. Natl. Acad. Sci. USA 89:11357- 11341 (1992) (said references incorporated by reference in their entireties).

[0222] Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), such methods can be used to generate antibodies with altered activity (*e.g.*, antibodies with higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten *et al.*, Curr. Opinion Biotechnol. 8:724-35 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, *et al.*, J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, polynucleotides encoding antibodies of the invention may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more portions of a polynucleotide encoding an antibody which portions specifically bind to PA may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[0223] Moreover, the antibodies of the present invention (including antibody fragments or variants thereof), can be fused to marker sequences, such as a polypeptides to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine polypeptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, Cell 37:767 (1984)) and the FLAG® tag (Stratagene, La Jolla, CA).

[0224] The present invention further encompasses antibodies (including antibody fragments or variants thereof), conjugated to a diagnostic or therapeutic agent. The antibodies can be used, for example, as part of a clinical testing procedure to, *e.g.*, determine the safety or efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include, but are not limited to, various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include, but are not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include, but are not limited to, streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include, but are not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes, but is not limited to, luminol; examples of bioluminescent materials include, but are not limited to, luciferase, luciferin, and aequorin; and examples of suitable radioactive material include, but are not limited to, iodine (^{121}I, ^{123}I, ^{125}I, ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{111}In, ^{112}In, ^{113m}In, ^{115m}In), technetium (^{99}Tc,^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum

(^{99}Mo), xenon (^{135}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, and ^{97}Ru.

[0225] Further, an antibody of the invention (including an scFv or other molecule comprising, or alternatively consisting of, antibody fragments or variants thereof), may be coupled or conjugated to a therapeutic moiety such as a cytotoxin, *e.g.*, a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, *e.g.*, alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{135}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Y, ^{117}Tin, ^{186}Re, ^{188}Re and ^{166}Ho. In specific embodiments, an antibody or fragment thereof is attached to macrocyclic chelators that chelate radiometal ions, including but not limited to, ^{177}Lu, ^{90}Y, ^{166}Ho, and ^{153}Sm, to polypeptides. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the an antibody of the invention or fragment thereof via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90, 1998; Peterson et al., Bioconjug. Chem. 10(4):553-7, 1999; and Zimmerman et al., Nucl. Med. Biol. 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety.

[0226] A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include, but are not limited to, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, thymidine kinase, endonuclease, RNAse, and puromycin and fragments, variants or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[0227] Techniques known in the art may be applied to label antibodies of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,711; 5,696,239; 5,652,371; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety) and direct coupling reactions (e.g., Bolton-Hunter and Chloramine-T reaction).

[0228] The antibodies of the invention which are conjugates can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, but are not limited to, for example, a toxin such as abrin, ricin A, alpha toxin, pseudomonas exotoxin, or diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, *e.g.*, TNF-alpha, TNF-beta, AIM I (see, International Publication No. WO 97/35899), AIM II (see, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol.,* 6:1567-1574 (1994)), VEGI (see, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, *e.g.*, angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 (IL- 1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), or other growth factors.

[0229] Antibodies of the invention (including antibody fragments or variants thereof), may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[0230] Techniques for conjugating a therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera

et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

[0231] Alternatively, an antibody of the invention can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[0232] An antibody of the invention (including an other molecules comprising, or alternatively consisting of, an antibody fragment or variant thereof), with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Uses of Antibodies of the Invention

[0233] Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of PA polypeptides in biological and non-biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety). By way of another non-limiting example, antibodies of the invention may be administered to individuals as a form of passive immunization.

[0234] Prophylactic or therapeutic treatment with anti-PA antibodies has advantages over other anti-anthrax agents, such as antibiotics, in that anti-PA antibodies provide protection against drug resistant strains; anti-PA antibodies can be given as either a single dose treatment or can be given in multiple doses (e.g., bi-weekly or monthly dosing); individual doses of anti-PA antibodies will have a relatively long duration of effect; can be administered subcutaneously in addition to other routes of administration (e.g., intravenously), and will be useful in re-exposure or flare situations. Given that the anti-PA antibodies provided herein are fully human antibodies, the risk of side effects due to anti-PA treatment will be minimal when administered as fully human antibodies.

Epitope Mapping

[0235] The present invention provides antibodies (including antibody fragments or variants thereof), that can be used to identify epitopes of a PA polypeptide (e.g., SEQ ID NO:2)) using techniques described herein or otherwise known in the art. Fragments which function as epitopes may be produced by any conventional means. (See, *e.g.*, Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,711,211.) Identified epitopes of antibodies of the present invention may, for example, be used as vaccine candidates, i.e., to immunize an individual to elicit antibodies against the naturally occurring forms of PA polypeptides.

Diagnostic Uses of Antibodies

[0236] Labeled antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) which specifically bind to a PA polypeptide can be used for diagnostic purposes to detect, diagnose, prognose, or monitor the presence of the intact *Bacillus anthracis* spore or organism, or simply the components of anthrax toxin. In specific embodiments, labeled antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) which specifically bind to a PA polypeptide can be used for diagnostic purposes to detect, diagnose, prognose, or monitor the course of anthrax infection.

[0237] The invention provides for the detection of expression of a PA polypeptide comprising: (a) assaying the expression of a PA polypeptide in a (biological – or non-biological) sample from an individual using one or more antibodies of the invention that specifically binds to PA; and (b) detecting the presence of PA polypeptide in the sample.

[0238] The invention provides for the detection of aberrant expression of a PA polypeptide comprising: (a) assaying the expression of a PA polypeptide in from one strain of *Bacillus anthracis* using one or more antibodies of the invention that specifically binds to PA; and (b) comparing the level of a PA polypeptide in the biological sample with a standard level of a PA polypeptide, *e.g.*, in a reference strain of *Bacillus anthracis*, whereby an increase or decrease in the assayed level of a PA polypeptide compared to the standard level of a PA polypeptide is indicative of aberrant expression.

[0239] By "biological sample" is intended any fluids and/or cells obtained from an individual, body fluid, body tissue, body cell, cell line, tissue culture, bacterial culture, or other source which may contain a PA polypeptide protein or mRNA. Body fluids include,

but are not limited to, sera, plasma, urine, synovial fluid, pleural fluid, edema fluid, spinal fluid, saliva, and mucous. Tissues samples may be taken from virtually any tissue in the body. Tissue samples may also be obtained from autopsy material. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0240] One aspect of the invention is the detection and diagnosis of a disease or disorder associated with *Bacillus anthracis* or anthrax toxins in an animal, preferably a mammal and most preferably a human.

Therapeutic and Prophylactic Uses of Antibodies

[0241] One or more antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that specifically bind to PA may be used locally or systemically in the body as a prophylactic or a therapeutic. The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) to an animal, preferably a mammal, and most preferably a human, for treating individuals infected with *Bacillus anthracis* bacteria and/or *B. anthracis* spores or individuals that have been exposed to *B. anthracis* bacteria, *B. anthracis* spores and/or anthrax toxins. Anthrax infection occurs when an animal has *B. anthracis* bacteria and/or *B. anthracis* spores within its body or in contact with the surface of its body. An animal may be considered as poisoned with anthrax toxin when it has within its body or in contact with the surface of its body, lethal toxin, edema toxin, lethal factor or edema factor.

[0242] Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention and nucleic acids encoding antibodies of the invention as described herein. The treatment and/or prevention of anthrax infection and/or anthrax toxin poisoning includes alleviating or preventing symptoms associated with anthrax infection and/or anthrax toxin poisoning.

[0243] For example, bacteremia occurs in almost all cases of anthrax that progress to a fatal outcome. Antibodies of the invention may be used to prevent the development of bacteremia in anthrax patients or to treat patients that have developed bacteremia associated with anthrax infection. In specific embodiments, anti-PA antibodies of the invention which activate the complement cascade, i.e. IgG1, IgG2, IgG3, and IgA1 and

IgM antibodies, are used to prevent the development of bacteremia in anthrax patients or to treat patients that have developed bacteremia associated with anthrax infection.

[0244] In other embodiments, antibodies of the invention may have a bactericidal and or bacteriostatic effect on *B. anthracis* bacteria. By way of non-limiting example, antibodies of the invention may activate the classical complement pathway and/or enhance the activation of the alternative complement pathway. Alternatively, antibodies of the invention may opsonize *B. anthracis* bacteria. Opsonized bacteria then may be a target for antibody dependent cell-mediated cytotoxicty (ADCC). In another embodiment, antibodies of the invention may catalyze the generation of hydrogen peroxide from singlet molecular oxygen and water which chemical reaction results in the efficient killing of bacteria. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[0245] Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

Therapeutic/Prophylactic Compositions and Administration

[0246] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of antibody (or fragment or variant thereof) or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, an antibody or fragment or variant thereof is substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to, animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably a human.

[0247] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[0248] Various delivery systems are known and can be used to administer antibody or fragment or variant thereof of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem.

262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0249] The preferred route of administration for antibodies of the invention will depend, in part, on the time of administration relative to the time of exposure or potential exposure to anthrax bacterium, anthrax spores, and/or anthrax derived toxins such as lethal toxin and edema toxin. For example, if administration of the antibody to an individual occurs after an actual or suspected exposure to anthrax bacterium, anthrax spores, and/or anthrax derived toxins, it would be most expedient to deliver the antibodies via a route which will provide the quickest time to maximum concentration (Tmax) and/or the greatest maximum concentration (Cmax) of serum anti-PA antibody levels. The shortest Tmax in serum is achieved using intravenous administration, because the antibody is delivered directly to the serum. It has also been shown in pharmacokinetic studies using rabbits and cynomolgus monkeys, that intramuscular administration results in a slightly higher Cmax and a slightly faster Tmax as compared to subcutaneous administration. Thus, post-exposure administration of antibodies of the invention is preferably performed intravenously. However, due to the time, materials and facilities required for intravenous administration, other routes of administration (such as intramuscular or subcutaneous administration) may be preferable for post-exposure administration of anti-PA antibodies especially in mass exposure events, exposure events in isolated areas or in battlefield conditions, or other similar situations.

[0250] On the other hand, if the time the antibody stays in the body (residence time) is the greatest clinical consideration, it may be preferable to administer the antibody

via a route that provides for a relatively long terminal half life of the antibody in serum and a long residence time. If antibodies are being administered prophylactically, prior to exposure or potential exposure to anthrax bacterium, anthrax spores, and/or anthrax derived toxins, it would be desirable to ensure the greatest longevity of the efficacy of the prophylactic antibody treatment by administering the antibodies via the route that provides for a relatively long terminal half life of the antibody in serum and a long residence time. It has been shown in pharmacokinetic studies using rabbits and cynomolgus monkeys, that that intramuscular or subcutaneous administration gives a longer terminal half life and/or residence time compared to intravenous administration. Thus, pre-exposure administration of anti-PA antibodies of the invention is preferably performed intramuscularly or subcutaneously.

[0251] In a preferred embodiment the antibody of the invention is formulated in 10 mM sodium citrate, 1.8% glycine, 1.0% sucrose, 0.02% polysorbate 80 (w/v), pH 6.5. In another preferred embodiment, the antibody of the invention is formulated in 10 mM sodium citrate, 1.8% glycine, 1.0% sucrose, 0.02% polysorbate 80 (w/v), pH 6.5 for subcutaneous, intramuscular and/or intravenous administration. Of course, any formulation suitable for clinical administration may be used.

[0252] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[0253] In another embodiment, the composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1535 (1990); Treat *et al.*, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327; see generally ibid.).

[0254] In yet another embodiment, the composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC

Crit. Ref. Biomed. Eng. 14:20 1 (1987); Buchwald *et al.*, Surgery 88:507 (1980); Saudek *et al.*, N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:71 (1983); see also Levy *et al.*, Science 228:190 (1985); During *et al.*, Ann. Neurol. 25:35 1 (1989); Howard *et al.*, J. Neurosurg. 7 1:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[0255] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1535 (1990)).

[0256] In a specific embodiment where the composition of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot *et al.*, Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0257] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of an antibody or a fragment thereof, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil,

soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the antibody, or fragment thereof, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[0258] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0259] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived

from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0260] The amount of the composition of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0261] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 50 mg/kg of the patient's body weight. In specific embodiments, the dosage administered to a patient is exactly or about 1 mg/kg of the patient's body weight. In specific embodiments, the dosage administered to a patient is exactly or about 3 mg/kg of the patient's body weight. In specific embodiments, the dosage administered to a patient is exactly or about 5 mg/kg of the patient's body weight. In specific embodiments, the dosage administered to a patient is exactly or about 10 mg/kg of the patient's body weight. In specific embodiments, the dosage administered to a patient is exactly or about 20 mg/kg of the patient's body weight. In specific embodiments, the dosage administered to a patient is exactly or about 30 mg/kg of the patient's body weight. In specific embodiments, the dosage administered to a patient is exactly or about 40 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of therapeutic or pharmaceutical compositions of the invention may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the antibodies by modifications such as, for example, lipidation.

[0262] Because bacteria and/or toxin will already be present when a patient is to be treated therapeutically, therapeutic dosages generally will be greater than prophylactic

dosages. In specific embodiments, a therapeutic dosage of anti-PA antibodies of the invention will be in the range of 1 to 100 mg/kg of the patient's body weight. In preferred embodiments, a therapeutic dosage of anti-PA antibodies of the invention will be in the range of 10 to 40 mg/kg of the patient's body weight. In specific preferred embodiments, a therapeutic dosage of anti-PA antibodies of the invention will be exactly or about 10 mg/kg of the patient's body weight. In specific preferred embodiments, a therapeutic dosage of anti-PA antibodies of the invention will be exactly or about 20 mg/kg of the patient's body weight. In specific preferred embodiments, a therapeutic dosage of anti-PA antibodies of the invention will be exactly or about 30 mg/kg of the patient's body weight. In specific preferred embodiments, a therapeutic dosage of anti-PA antibodies of the invention will be exactly or about 40 mg/kg of the patient's body weight.

[0263] In specific embodiments, a prophylactic dose of anti-PA antibodies of the invention will be in the range of 0.1 to 20 mg/kg of the patient's body weight. In preferred embodiments, a prophylactic dose of anti-PA antibodies of the invention will be in the range of 1 to 10 mg/kg of the patient's body weight. In specific preferred embodiments, a prophylactic dose of anti-PA antibodies of the invention will be exactly or about 1 mg/kg of the patient's body weight. In specific preferred embodiments, a prophylactic dose of anti-PA antibodies of the invention will be exactly or about 3 mg/kg of the patient's body weight. In specific preferred embodiments, a prophylactic dose of anti-PA antibodies of the invention will be exactly or about 5 mg/kg of the patient's body weight. In specific preferred embodiments, a prophylactic dose of anti-PA antibodies of the invention will be exactly or about 10 mg/kg of the patient's body weight.

[0264] Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments, or variants, (e.g., derivatives), or nucleic acids, are administered to a human patient for therapy or prophylaxis.

[0265] It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that specifically bind to PA, or polynucleotides encoding antibodies that specifically bind to PA, for both immunoassays and administration to patients. Such antibodies will preferably have an affinity for PA and/or PA polypeptide fragments. Preferred binding affinities include those with a dissociation constant or K_D of less than or equal to 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M,

5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, or 10^{-5} M. More preferably, antibodies of the invention bind PA polypeptides or fragments or variants thereof with a dissociation constant or K_D less than or equal to 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, or 10^{-8} M. Even more preferably, antibodies of the invention bind PA polypeptides or fragments or variants thereof with a dissociation constant or K_D less than or equal to 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, $5 \times ^{-13}$ M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0266] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[0267] The antibody and antibody compositions of the invention may be administered alone or in combination with other therapeutic agents, including but not limited to antibiotics, antivirals, anti-retroviral agents, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents and cytokines. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0268] In some embodiments, antibodies of the invention that are administered to an animal, preferably a human, for therapeutic or prophylactic uses are multimeric antibodies. In specific embodiments, antibodies of the invention are homodimeric IgG molecules. In other specific embodiments, antibodies of the invention are homodimeric IgG1 molecules. In specific embodiments, antibodies of the invention are homotrimeric IgG molecules. In other specific embodiments, antibodies of the invention are trimeric IgG1

molecules. In other specific embodiments, antibodies of the invention are higher-order multimers of IgG molecules (e.g., tetramers, penatmers and hexamers]. In still further specific embodiments, antibodies of the IgG molecules comprising the higher order multimers of IgG molecules are IgG1 molecules.

[0269] Alternatively, antibodies of the invention for therapeutic or prophylactic uses may be administered in combination with crosslinking agents known in the art, including but not limited to anti-IgG antibodies.

Combination Administration with antibiotics, other anti-anthrax agents, or other anti-bioterorrism agents

[0270] The antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) may be administered alone or in combination with other therapeutic or prophylactic regimens (*e.g.*, radiation therapy, chemotherapy, hormonal therapy, immunotherapy, anti-tumor agents, anti-angiogenesis and anti-inflammatory agents). In specific embodiments, antibodies of the invention are administered in combination with one or more anti-anthrax agents. An anti-anthrax agent is a substance that is used to treat or prevent anthrax infection and/or anthrax toxin-poisoning. Such combinatorial therapy may be administered sequentially and/or concomitantly.

[0271] The antibodies of the invention may be administered prophylactically or therapeutically. It is specifically contemplated that the antibodies of the invention may be administered to provide protection against anthrax infection and/or anthrax toxin exposure as a supplementary or supportive measure in addition to other prophylactic or therapeutic regimens. For example, conventional treatment for known or suspected anthrax infection and/or anthrax toxin exposure typically includes immunization with an anthrax vaccine and/or administration of antibiotics. However, it takes significant time both for an individual or animal to build up antibody titers against anthrax following immunization, and for antibiotic treatment regimens to effectively control an anthrax infection. During these time periods, conventional treatment does little to offset the clinical effect of anthrax toxins on the patient. In one embodiment of the invention, the antibodies of the invention may be administered as a form of passive immunization or supportive therapy during the time period following immunization with an anthrax vaccine, in order to prevent or lessen the effect of anthrax toxins prior to development of protective levels of anti-anthrax

antibody titers. Another exemplary use of the antibodies of the invention is as supportive or supplemental therapy for an individual undergoing antibiotic treatment for anthrax exposure, in order to prevent or lessen the effect of anthrax toxins while the antibiotic treatment regimen is given time to eliminate the anthrax infection.

[0272] In specific embodiments, anti-PA antibodies of the invention may be administered in combination with other anti-PA antibodies, or other antibodies reactive with different protein components of *Bacillus anthracis* or anthrax toxin components (including EF and LF).

[0273] In specific embodiments, anti-PA antibodies of the invention may be administered in combination with one or more antibiotic agents. In a particular embodiment, anti-PA antibodies of the invention may be administered in combination with the antibiotic Ciprofloxacin Hydrochloride (Cipro). In other embodiments, anti-PA antibodies of the invention may be administered in combination with the antibiotic doxycycline. In other embodiments, anti-PA antibodies of the invention may be administered in combination with the antibiotic penicillin G procaine. In other embodiments, anti-PA antibodies of the invention may be administered in combination with the antibiotic amoxicillin. In other embodiments, anti-PA antibodies of the invention may be administered in combination with the antibiotic ofloxacin. In other embodiments, anti-PA antibodies of the invention may be administered in combination with the antibiotic penicillin levofloxacin.

[0274] Other antibiotics that may be administered in combination with anti-PA antibodies of the invention include, but are not limited to, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, erythromycin, fluoroquinolones, macrolides, metronidazole, cephalothin, cefazolin, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, imipenem, clarithromycin, gentamycin,and vancomycin.

[0275] In specific embodiments, antibodies of the invention are administered in combination with other therapeutics or prophylactics such as a soluble form of an anthrax receptor (e.g., SEQ ID NO:3 described in Nature (2002) 414:225-229 which is hereby incorporated by reference in its entirety, e.g., a polypeptide comprising amino acids 1 to 227 of 41-227 SEQ ID NO:3), a soluble for of the CMG2 receptor (described in Scobie et al., *Proceedings of the National Academy of Sciences USA* (2003) 100:5170-5174 which is hereby incorporatedby reference in its entirety) or anti-ATR or anti-CMG2 antibodies that

block binding of PA to ATR or CMG2, respectively. Other therapeutics or prophylactics that may be administered in combination with an antibody of the present invention include mutant forms of PA such as the EF/LF translocation deficient forms of PA described in International Publication Number WO01/82788 and in Science (2001) 292:695-697, both of which are hereby incorporated by reference in their entireties. Other therapeutics or prophylactics that may be administered in combination with an antibody of the present invention include peptide inhibitors that block LF binding to PA such as the P1 peptide, or its polyvalent form described in Nature Biotechnology (2002) 19:958-961 which is hereby incorporated by reference in its entirety. Still other therapeutics or prophylactics that may be administered in combination with an antibody of the present invention include, but are not limited to antibiotics, anthrax vaccines, antibodies immunoreactive with LF, EF or other protein moieties of *Bacillus anthracis*.

[0276] In specific embodiments, antibodies of the invention are administered in combination with anthrax vaccines, including but not limited to vaccines such as Anthrax Vaccine Adsorbed (AVA, also known as MDPH-PA and BioThrax), manufactured by BioPort Corporation, Lansing, Michigan; the vaccine manufactured by the Center for Applied Microbiology & Research (CAMR), Porton Down, Salisbury, England; vaccines utilizing recombinantly expressed anthrax PA, LF and/or EF proteins, whether wild-type or mutated (including both naturally occurring and artificially generated mutants); and other anthrax vaccines, including live, modified live, and killed vaccines.

[0277] In another specific embodiment, antibodies and antibody compositions of the invention are administered in combination with protease inhibitors. In specific embodiments, antibodies and antibody compositions of the invention are administered in combination with furin inhibitors.

Additional Combination Therapies

[0278] In other embodiments, antibody and antibody compositions of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the antibody and antibody compositions of the invention, include, but are not limited to, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™,

FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, antibody and antibody compositions of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic cytomegalovirus infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat, prevent, and/or diagnose an opportunistic fungal infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, antibody and antibody compositions of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat, prevent, and/or diagnose an opportunistic bacterial infection.

[0279] In a further embodiment, the antibody and antibody compositions of the invention are administered in combination with an antiviral agent. Antiviral agents that

may be administered with the antibody and antibody compositions of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0280] In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

[0281] In a specific embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the compositions of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin (adriamycin), bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane,

vincristine sulfate, vinblastine sulfate, etoposide, Topotecan, 5-Fluorouracil, paclitaxel (Taxol), Cisplatin, Cytarabine, and IFN-gamma, irinotecan (Camptosar, CPT-11), irinotecan analogs, and gemcitabine (GEMZAR™)).

[0282] In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, antibody and antibody compositions of the invention are administered in combination with Rituximab. In a further embodiment, antibody and antibody compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of the components of CHOP.

[0283] In additional preferred embodiments, the compositions of the invention are administered in combination with TRAIL polypeptides or fragments or variants thereof, particularly of the extracellular soluble domain of TRAIL.

[0284] In one embodiment, the compositions of the invention are administered in combination with other members of the TNF family or antibodies specific for TNF receptor family members. In specific embodiments Antibodies and antibody compositions of the invention are administered in combination with anti-TNF-alpha and/ or anti-IL-1Beta antibodies. TNF, TNF-related or TNF-like molecules that may be administered with the compositions of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), TRAIL, AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/35904), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892),TR10 (International Publication No. WO 98/54202),312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

[0285] In a more preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with an antimalarial, methotrexate, anti-TNF antibody, ENBREL™ and/or suflasalazine. In one embodiment, the antibody and antibody compositions of the invention are administered in combination with methotrexate. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with anti-TNF antibody. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with methotrexate and anti-TNF antibody. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with suflasalazine. In another specific embodiment, the antibody and antibody compositions of the invention are administered in combination with methotrexate, anti-TNF antibody, and suflasalazine. In another embodiment, the antibody and antibody compositions of the invention are administered in combination ENBREL™. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with ENBREL™ and methotrexate. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In another embodiment, the antibody and antibody compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In other embodiments, one or more antimalarials is combined with one of the above-recited combinations. In a specific embodiment, the antibody and antibody compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL™, methotrexate and suflasalazine. In another specific embodiment, the antibody and antibody compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), sulfasalazine, anti-TNF antibody, and methotrexate.

[0286] Conventional nonspecific immunosuppressive agents, that may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs cyclophosphamide, cyclophosphamide IV, methylprednisolone, prednisolone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

[0287] In specific embodiments, antibody and antibody compositions of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0288] In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with steroid therapy. Steroids that may be administered in combination with the antibody and antibody compositions of the invention, include, but are not limited to, oral corticosteroids, prednisone, and methylprednisolone (e.g., IV methylprednisolone). In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with prednisone. In a further specific embodiment, the antibody and antibody compositions of the invention are administered in combination with prednisone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the antibody and antibody compositions of the invention and prednisone are those described herein, and include, but are not limited to, azathioprine, cylophosphamide, and cyclophosphamide IV. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with methylprednisolone. In a further specific embodiment, the antibody and antibody compositions of the invention are administered in combination with methylprednisolone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the antibody and antibody compositions of the invention and methylprednisolone are those described herein, and include, but are not limited to, azathioprine, cylophosphamide, and cyclophosphamide IV.

[0289] The invention also encompasses combining the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) with other proposed or conventional hematopoietic therapies. Thus, for example, the polynucleotides and/or polypeptides of the invention (and/or agonists or antagonists thereof) can be combined with compounds that singly exhibit erythropoietic stimulatory effects, such as erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, and triiodothyzonine. Also

encompassed are combinations of the antibody and antibody compositions of the invention with compounds generally used to treat aplastic anemia, such as, for example, methenolene, stanozolol, and nandrolone; to treat iron-deficiency anemia, such as, for example, iron preparations; to treat malignant anemia, such as, for example, vitamin B_{12} and/or folic acid; and to treat hemolytic anemia, such as, for example, adrenocortical steroids, e.g., corticoids. See e.g., Resegotti et al., Panminerva Medica, 23:243-248 (1981); Kurtz, FEBS Letters, 14a:105-108 (1982); McGonigle et al., Kidney Int., 25:437-444 (1984); and Pavlovic-Kantera, Expt. Hematol., 8(supp. 8) 283-291 (1980), the contents of each of which are hereby incorporated by reference in their entireties.

[0290] Compounds that enhance the effects of or synergize with erythropoietin are also useful as adjuvants herein, and include but are not limited to, adrenergic agonists, thyroid hormones, androgens, hepatic erythropoietic factors, erythrotropins, and erythrogenins, See for e.g., Dunn, "Current Concepts in Erythropoiesis", John Wiley and Sons (Chichester, England, 1983); Kalmani, Kidney Int., 22:383-391 (1982); Shahidi, New Eng. J. Med., 289:72-80 (1973); Urabe et al., J. Exp. Med., 149:1314-1325 (1979); Billat et al., Expt. Hematol., 10:135-140 (1982); Naughton et al., Acta Haemat, 69:171-179 (1983); Cognote et al. in abstract 364, Proceedings 7th Intl. Cong. of Endocrinology (Quebec City, Quebec, July 1-7, 1984); and Rothman et al., 1982, J. Surg. Oncol., 20:105-108 (1982). Methods for stimulating hematopoiesis comprise administering a hematopoietically effective amount (i.e., an amount which effects the formation of blood cells) of a pharmaceutical composition containing polynucleotides and/or polypeptides of the invention to a patient. The polynucleotides and/or polypeptides of the invention are administered to the patient by any suitable technique, including but not limited to, parenteral, sublingual, topical, intrapulmonary and intranasal, and those techniques further discussed herein. The pharmaceutical composition optionally contains one or more members of the group consisting of erythropoietin, testosterone, progenitor cell stimulators, insulin-like growth factor, prostaglandins, serotonin, cyclic AMP, prolactin, triiodothyzonine, methenolene, stanozolol, and nandrolone, iron preparations, vitamin B_{12}, folic acid and/or adrenocortical steroids.

[0291] In an additional embodiment, the antibody and antibody compositions of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the antibody and antibody

compositions of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

[0292] In an additional embodiment, the antibody and antibody compositions of the invention are administered alone or in combination with an anti-angiogenic agent(s). Anti-angiogenic agents that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

[0293] Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

[0294] Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

[0295] Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

[0296] A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents Actions 36:312-316, 1992); and metalloproteinase inhibitors such as BB94.

[0297] Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolmidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC359555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3540) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

[0298] Anti-angiogenic agents that may be administered in combination with the compounds of the invention may work through a variety of mechanisms including, but not

limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, AG-3540 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, EMD-121974 (Merck KcgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the antibody and antibody compositions of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

[0299] In particular embodiments, the use of antibody and antibody compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of cancers and other hyperproliferative disorders.

[0300] In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), biologically active fragments, variants, or derivatives of

CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0301] In another embodiment, antibody and antibody compositions of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, heparin, warfarin, and aspirin. In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with warfarin. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with warfarin and aspirin. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with heparin. In another specific embodiment, antibody and antibody compositions of the invention are administered in combination with heparin and aspirin.

[0302] In another embodiment, antibody and antibody compositions of the invention are administered in combination with an agent that suppresses the production of anticardiolipin antibodies. In specific embodiments, the polynucleotides of the invention are administered in combination with an agent that blocks and/or reduces the ability of anticardiolipin antibodies to bind phospholipid-binding plasma protein beta 2-glycoprotein I (b2GPI).

[0303] In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with an antimalarial. Antimalarials that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, hydroxychloroquine, chloroquine, and/or quinacrine.

[0304] In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with an NSAID.

[0305] In a nonexclusive embodiment, the antibody and antibody compositions of the invention are administered in combination with one, two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecasermin (Chiron), T-714 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000 (Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-1Ra gene

therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIIL-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-671 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

[0306] In a preferred embodiment, the antibody and antibody compositions of the invention are administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL™ (Etanercept), anti-TNF antibody, LJP 394 (La Jolla Pharmaceutical Company, San Diego, California) and prednisolone.

[0307] In an additional embodiment, antibody and antibody compositions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the antibody and antibody compositions of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, antibody and antibody compositions of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0308] CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), biologically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g., agonistic or antagonistic antibodies).

[0309] In an additional embodiment, the antibody and antibody compositions of the invention are administered in combination with cytokines. Cytokines that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, GM-CSF, G-CSF, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-alpha, IFN-beta, IFN-gamma, TNF-alpha, and TNF-beta. In preferred embodiments, antibody and antibody compositions of the invention are administered with TRAIL receptor. In another embodiment, antibody and antibody compositions of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10,

IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, and IL-22. In preferred embodiments, the antibody and antibody compositions of the invention are administered in combination with IL4 and IL10.

[0310] In one embodiment, the antibody and antibody compositions of the invention are administered in combination with one or more chemokines. In specific embodiments, the antibody and antibody compositions of the invention are administered in combination with an α(CxC) chemokine selected from the group consisting of gamma-interferon inducible protein-10 (γIP-10), interleukin-8 (IL-8), platelet factor-4 (PF4), neutrophil activating protein (NAP-2), GRO-α, GRO-β, GRO-γ, neutrophil-activating peptide (ENA-78), granulocyte chemoattractant protein-2 (GCP-2), and stromal cell-derived factor-1 (SDF-1, or pre-B cell stimulatory factor (PBSF)); and/or a β(CC) chemokine selected from the group consisting of: RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1 beta (MIP-1β), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-2 (MCP-2), monocyte chemotactic protein-3 (MCP-3), monocyte chemotactic protein-4 (MCP-4) macrophage inflammatory protein-1 gamma (MIP-1γ), macrophage inflammatory protein-3 alpha (MIP-3α), macrophage inflammatory protein-3 beta (MIP-3β), macrophage inflammatory protein-4 (MIP-4/DC-CK-1/PARC), eotaxin, Exodus, and I-309; and/or the γ(C) chemokine, lymphotactin.

[0311] In another embodiment, the antibody and antibody compositions of the invention are administered with chemokine beta-8, chemokine beta-1, and/or macrophage inflammatory protein-4. In a preferred embodiment, the antibody and antibody compositions of the invention are administered with chemokine beta-8.

[0312] In an additional embodiment, the antibody and antibody compositions of the invention are administered in combination with an IL-4 antagonist. IL-4 antagonists that may be administered with the antibody and antibody compositions of the invention include, but are not limited to: soluble IL-4 receptor polypeptides, multimeric forms of soluble IL-4 receptor polypeptides; anti-IL-4 receptor antibodies that bind the IL-4 receptor without transducing the biological signal elicited by IL-4, anti-IL4 antibodies that block binding of IL-4 to one or more IL-4 receptors, and muteins of IL-4 that bind IL-4 receptors but do not transduce the biological signal elicited by IL-4. Preferably, the

antibodies employed according to this method are monoclonal antibodies (including antibody fragments, such as, for example, those described herein).

[0313] In an additional embodiment, the antibody and antibody compositions of the invention are administered in combination with fibroblast growth factors. Fibroblast growth factors that may be administered with the antibody and antibody compositions of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

Demonstration of Therapeutic or Prophylactic Utility of a Composition

[0314] The compounds of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays which can be used to determine whether administration of a specific antibody or composition of the present invention is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered an antibody or composition of the present invention, and the effect of such an antibody or composition of the present invention upon the tissue sample is observed. In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if an antibody or composition of the present invention has a desired effect upon such cell types. Preferably, the antibodies or compositions of the invention are also tested in *in vitro* assays and animal model systems prior to administration to humans.

[0315] Antibodies or compositions of the present invention for use in therapy can be tested for their toxicity in suitable animal model systems, including but not limited to rats, mice, chicken, cows, monkeys, and rabbits. For *in vivo* testing of an antibody or composition's toxicity any animal model system known in the art may be used.

[0316] Antibodies or compositions of the invention can also be tested for their ability to reduce bacterial numbers in *in vitro* and *in vivo* assays known to those of skill in the art. Antibodies or compositions of the invention can also be tested for their ability to alleviate of one or more symptoms associated with anthrax disease or anthrax toxin poisoning. Antibodies or antibody compositions of the invention can also be tested for their ability to decrease the time course of the infectious disease. Further, antibodies or compositions of the invention can be tested for their ability to increase the survival period of animals suffering from anthrax or anthrax toxin poisoning. Techniques known to those of skill in

the art can be used to analyze the function of the antibodies or compositions of the invention *in vivo*.

[0317] Efficacy in treating or preventing bacterial (e.g. *Bacillus anthracis*) infection may be demonstrated by detecting the ability of an antibody or composition of the invention to inhibit the replication of the bacteria, to inhibit transmission or prevent the bacteria from establishing itself in its host, or to prevent, ameliorate or alleviate the symptoms of disease progression. The treatment is considered therapeutic if there is, for example, a reduction in bacterial load, amelioration of one or more symptoms, or a decrease in mortality and/or morbidity following administration of an antibody or composition of the invention.

Panels/Mixtures

[0318] The present invention also provides for mixtures of antibodies (including scFvs and other molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that specifically bind to PA or a fragment or variant thereof, wherein the mixture has at least one, two, three, four, five or more different antibodies of the invention. In specific embodiments, the invention provides mixtures of at least 2, preferably at least 4, at least 6, at least 8, at least 10, at least 12, at least 15, at least 20, or at least 25 different antibodies that specifically bind to PA or fragments or variants thereof, wherein at least 1, at least 2, at least 4, at least 6, or at least 10, antibodies of the mixture is an antibody of the invention. In a specific embodiment, each antibody of the mixture is an antibody of the invention.

[0319] The present invention also provides for panels of antibodies (including scFvs and other molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that specifically bind to PA or a fragment or variant thereof, wherein the panel has at least one, two, three, four, five or more different antibodies of the invention. In specific embodiments, the invention provides for panels of antibodies that have different affinities for PA, different specificities for PA, or different dissociation rates. The invention provides panels of at least 10, preferably at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, or at least 1000,

antibodies. Panels of antibodies can be used, for example, in 96 well plates for assays such as ELISAs.

[0320] The present invention further provides for compositions comprising, one or more antibodies (including molecules comprising, or alternatively consisting of antibody fragments or variants of the invention). In one embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VH domains of a one or more of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a variant thereof. In another embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VH CDR1s of a VH domain of one or more of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a variant thereof. In another embodiment, a composition of the present invention comprises, one, two, three, four, five or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VH CDR2s of a VH domain of one or more of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a variant thereof. In a preferred embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VH CDR3s as of a VH domain of one or more of the scFvs or recombinant antibodies expressed by the cell lines referred to in Table 1, or a variant thereof.

[0321] Other embodiments of the present invention providing for compositions comprising, one or more antibodies (including molecules comprising, or alternatively consisting of antibody fragments or variants of the invention) are listed below. In another embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternative consist of, a polypeptide having an amino acid sequence of any one or more of the VL domains of one or more of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a variant thereof. In another embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VL

CDR1s domains of one or more of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a variant thereof. In another embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VL CDR2s of one or more of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a variant thereof. In a preferred embodiment, a composition of the present invention comprises, one, two, three, four, five, or more antibodies that comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one or more of the VL CDR3s domains of one or more of the scFvs referred to in Table 1 or recombinant antibodies expressed by the cell lines referred to in Table 1, or a variant thereof.

Kits

[0322] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0323] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In an alterative embodiment, a kit comprises an antibody fragment that specifically binds to PA polypeptides or fragments or variants thereof. In a specific embodiment, the kits of the present invention contain a substantially isolated PA polypeptide or fragment or variant thereof as a control. Preferably, the kits of the present invention further comprise a control antibody which does not react with PA polypeptides or fragments or variants thereof. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to PA polypeptides (*e.g.*, the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate). In specific embodiments, the kit may include a recombinantly

produced or chemically synthesized PA polypeptide. The PA provided in the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above-described kit includes a solid support to which PA is attached. Such a kit may also include a non-attached reporter-labeled anti- human antibody. In this embodiment, binding of the antibody to PA can be detected by binding of the said reporter-labeled antibody.

[0324] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with PA polypeptides, and means for detecting the binding of PA polypeptides to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0325] In specific embodiments, a kit of the invention comprises a means for administering an antibody to an animal, preferably a human. Means for administering an antibody to an animal include a syringe.

Gene Therapy

[0326] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a anthrax or anthrax toxin poisoning, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[0327] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0328] For general reviews of the methods of gene therapy, see Goldspiel *et al.*, Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy *3:87-95* (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 1 *l(5):155-215* (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.),

Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[0329] In a preferred aspect, a composition of the invention comprises, or alternatively consists of, nucleic acids encoding an antibody, said nucleic acids being part of an expression vector that expresses the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra *et al.*, Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is an scFv; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments or variants thereof, of an antibody.

[0330] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

[0331] In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, *e.g.*, by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another

embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06 180; WO 92/22715; W092/203 16; W093/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra *et al.*, Nature 342:435-438 (1989)).

[0332] In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention or fragments or variants thereof are used. For example, a retroviral vector can be used (see Miller *et al.*, Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen *et al.*, Biotherapy 6:29 1-302 (1994), which describes the use of a retroviral vector to deliver the mdr 1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, J. Clin. Invest. 93:644-651(1994); Klein *et al.*, Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

[0333] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout *et al.*, Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, Science 252:431-434 (1991); Rosenfeld *et al.*, Cell 68:143- *155* (1992); Mastrangeli *et al.*, J. Clin. Invest.

91:225-234 (1993); PCT Publication W094/12649; and Wang, *et al.*, Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[0334] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh *et al.*, Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

[0335] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

[0336] In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell--mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, Meth. Enzymol. 217:599-718 (1993); Cohen *et al.*, Meth. Enzymol. 217:718-644 (1993); Clin. Pharma. Ther. 29:69-92m (1985)) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[0337] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[0338] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular

hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[0339] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[0340] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody or fragment thereof are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 7 1:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 71:771 (1986)).

[0341] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

Examples

Example 1: Isolation and characterization of scFvs referred to in Table 1

[0342] Maxisorp tubes (Nunc) were coated overnight with 10 micrograms/ml of PA83 protein in PBS at 4°C. Unbound PA was removed by washing the tubes with 1X PBST and 1X PBS followed by filling the tubes with a 3% milk solution in 1X PBS for one hour to block any exposed tube surface. Approximately 10^{13} TU of phage from phage display libraries available from Cambridge Antibody Technology (Cambridgshire, United Kingdom) diluted in 3%milk/1XPBS was applied to the tube and incubated for at least 60 minutes at room temperature. Tubes were washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine with gentle shaking after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage were then used to infect 10 ml of mid-log *E. coli* TG1 by incubating eluted phage with bacteria for 30 minutes at 37^0C. The *E. coli* were then plated on 2XYT plates containing 2% glucose and 100 micrograms/ml ampicillin. The resulting bacterial library was then rescued with delta gene 3 helper phage to prepare phage for a subsequent round

of selection. This process is usually repeated for a total of 2-4 rounds of affinity purification. Specific enrichment of PA binding phage can be monitored during the selection process. Individual clones from both the second and the third rounds of selections were screened for the ability to bind to PA protein using the assay protocol described below.

PA Binding Assay Protocol for scFv-Phage Library Screening

[0343] Purified full-length PA protein (PA83) was labeled with Biotin-LC-Sulfo - NHS (Pierce) at a molar challenge ratio of 8:1 in PBS, 8.0 for 60 minutes at 23°C. Protein was separated from free label using a NAP 5 gel filtration column (Amersahm-Pharmacia Biotech) following manufacturer's protocol. A polyclonal antibody specific for the M13 phage coat (Amersham-Pharmacia Biotech) was labeled with the electrochemi-luminescent reporter Origen-TAG-NHS ((Ori-TAG), IGEN International, Inc.) at a molar challenge ratio of 5:1 in PBS, 8.0 buffer for 60 minutes at 23°C. Protein was separated from free label using a NAP 5 gel filtration column (Amersham-Pharmacia Biotech) following manufacturer's protocol. The amount of incorporated Origen –TAG label was determined by measuring the absorbance of the undiluted labeling reaction at 455 nm in a 1 cm cuvette and dividing by 13,700 (extinction coefficient of Ori-TAG label) to obtain the Ori-TAG label concentration in moles per liter. This number was divided by the moles per liter IgG concentration in the labeling reaction. Label concentrations used in the assay ranged from 3 to 5 labels per IgG molecule. The biotinylated-PA83 and Origen-TAG labeled anti-M13 antibody were used to screen phage clones for PA binding as described below.

[0344] Individual *E. coli* colonies containing phagemid were inoculated into 96 well plates containing 100 microliters 2XTY+ 100 micrograms/ml ampicillin+ 2% glucose per well. Plates were incubated 37C for 4 hours, shaking. M13K07 helper phage was added to each well at a multiplicity of infection (MOI) of 2 to10 and the plates were incubated for a further 1 hour at 37C. The plates were centrifuged in a benchtop centrifuge at 2000 rpm for 10 minutes. The supernatant was removed and cell pellets were resuspended in 100 microliters 2XTY+ 100 micrograms/ml ampicillin + 50 micrograms/ml kanamycin and incubated at 30C overnight, with vigorous shaking. The next day, plates were centrifuged

at 2000 rpm for 10 min and 100 microliters of phage-containing supernatant from each well carefully transferred into a fresh 96-well plate.

[0345] The supernatants containing scFv-phage were screened for binding to PA83 using the following protocol: In a 96 well plate, 5 microliters of scFv-phage were combined with 150 microliters of 0.5 micrograms/ml Biotin-PA83 and 0.5 micrograms/ml Origen-Tag labeled anti-M13 polyclonal antibody and 20 micrograms of Streptavidin coated magnetic beads (Dynal M280 beads). The plate was sealed and mixed vigorously for 60 minutes at room temperature. The electrochemiluminescent (ECL) signal was measured in each well of the plate using an Origen M8 series ECL analyzer (IGEN International, Inc). Wells that showed ECL signals that were 5-fold above the assay background were scored as positive PA binders and submitted for sequencing.

[0346] The complete nucleotide sequence of the scFv insert from 980 PA positive binding phage clones was determined and a numerical summary of the sequence diversity and ability to bind PA in the above-described-PA Binding Assay is presented in the Table 4 below.

Table 4: Summary of ScFv Groups and CDR 3 Sequences for PA-Binding Phage Clones

Group	HC group, LC group	Representative scFv clone	HC CDR 3 Seq.	LC CDR 3 Seq.	PA Binding Pos/Neg
1	HC group 115, LC group 122:	PWB2001	HSPGDYAFDY	ASWDDSLNGRV	+
2	HC group 115, LC group 135:	PWB2855	HSPGDYAFDY	ASWDDSLKSRV	+
3	HC group 115, LC group 137:	PWB2916	HSPGDYAFDY	ASWDDSVNGRV	+
4	HC group 116, LC group 123:	PWB2002	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
5	HC group 116, LC group 127:	PWB2175	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
6	HC group 116, LC group 131:	PWB2362	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
7	HC group 116, LC group 132:	PWB2447	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
8	HC group 116, LC group 134:	PWB2754	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
9	HC group 117, LC group 122:	PWB2006	HSPGDYAFDY	ASWDDSLNGRV	+
10	HC group 118, LC group 124:	PWB2008	HSPGDYAFDY	ASWDDSLNGRV	+
11	HC group 119, LC group 123:	PWB2016	ASYLSTSSSLDY	NSRDSSGNHVV	+
12	HC group 119, LC group 133:	PWB2562	ASYLSTSSSLDY	NSRDSSGNHVV	+

13	HC group 120, LC group 123:	PWB2018	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
14	HC group 121, LC group 125	PWB2043	DLDSSTIPHREYGMDV	HSRDSSGNHVL	+
15	HC group 122, LC group 123:	PWB2061	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
16	HC group 123, LC group 123:	PWB2144	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
17	HC group 124, LC group 126:	PWB2153	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
18	HC group 125, LC group 123:	PWB2202	ASNLSTSSSLDY	NSRDSSGNHVV	+
19	HC group 126, LC group 128:	PWB2216	SGSSWSHFDF	SSYTTRSTRV	+
20	HC group 127, LC group 129:	PWB2281	GSPTGDLNVDVFDY	NSRDSSGNHVV	+
21	HC group 128, LC group 130:	PWB2301	HSPGDYAFDY	ASWDDSLNGRV	+
22	HC group 129, LC group 122:	PWB2323	VRDIRPGDYAFDY	ASWDDSLNGRV	+
23	HC group 130, LC group 123:	PWB2325	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
24	HC group 131, LC group 123:	PWB2334	Not Determined	NSRDSSGNHVV	+
25	HC group 132, LC group 122:	PWB2341	HSPGDYAFDY	ASWDDSLNGRV	+
26	HC group 133, LC group 123:	PWB2353	ASYLSTSPSLDY	NSRDSSGNHVV	+
27	HC group 134, LC group 123:	PWB2363	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
28	HC group 135, LC group 122:	PWB2364	HSPGDYAFDY	ASWDDSLNGRV	+
29	HC group 136, LC group 123:	PWB2376	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
30	HC group 137, LC group 128:	PWB2435	SGSSWSHFDF	SSYTTRSTRV	+
31	HC group 138, LC group 123:	PWB2456	AGRRTQLPPRDFLFEH	NSRDSSGNHVV	+
32	HC group 139, LC group 122:	PWB2466	HSPGDYAFDY	ASWDDSLNGRV	+
33	HC group 140, LC group 123:	PWB2502	ASNLSTSPSLDY	NSRDSSGNHVV	+
34	HC group 141, LC group 123:	PWB2532	AGRRTQLQPIDFLFEY	NSRDSSGNHVV	+
35	HC group 142, LC group 123:	PWB2617	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
36	HC group 143, LC group 122:	PWB2756	HSPGDYAFDY	ASWDDSLNGRV	+
37	HC group 144, LC group 123:	PWB2849	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
38	HC group 145, LC group 136:	PWB2873	GSGYSGYDFPYYYGM DV	HSRDSSGNHVL	+
39	HC group 146, LC group 123:	PWB2878	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
40	HC group 147, LC group 123:	PWB2955	Not Determined	NSRDSSGNHVV	+
41	HC group 2, LC group 2:	PWC2008	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
42	HC group 2, LC group 163:	PWC2065	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
43	HC group 2, LC group 188:	PWC2963	AGRRTQLQPRDFLFEY	NSRDSSGNHVV	+
44	HC group 140, LC group 155:	PWC2002	DSSSGWFFIDY	QSYDSSLGGYVI	+

45	HC group 140, LC group 160:	PWC2043	ARDSSSGWFFIDY	QSYDSSLGGYVI	+
46	HC group 140, LC group 165:	PWC2302	DSSSGWFFIDY	QSYDSSLGGYVI	+
47	HC group 140, LC group 166:	PWC2308	DSSSGWFFIDY	QSYDSSLGGYVI	+
48	HC group 140, LC group 167:	PWC2310	DSSSGWFFIDY	QSYDSSLGGYVI	+
49	HC group 140, LC group 169:	PWC2361	DSSSGWFFIDY	QSYDSSLGGYVI	+
50	HC group 140, LC group 172:	PWC2461	DSSSGWFFIDY	QSYDSSLGGYVI	+
51	HC group 140, LC group 175:	PWC2616	DSSSGWFFIDY	QSYDSSLGGYVI	+
52	HC group 140, LC group 176:	PWC2632	DSSSGWFFIDY		+
53	HC group 140, LC group 179:	PWC2678	DSSSGWFFIDY	QSYDSSLGGYVI	+
54	HC group 140, LC group 183:	PWC2748	DSSSGWFFIDY	QSYDSSLGGYGI	+
55	HC group 141, LC group 156:	PWC2004	SRYSSSPFRGGLDV	HSYDSSISGGI	+
56	HC group 141, LC group 157:	PWC2007	SRYSSSPFRGGLDV	HSYDSSISGWI	+
57	HC group 141, LC group 158:	PWC2010	SRYSSSPFRGGLDV	HSYDSSISGWI	+
58	HC group 141, LC group 159:	PWC2021	SRYSSSPFRGGLDV	HSYDSSIRGWI	+
59	HC group 141, LC group 161:	PWC2046	SRYSSSPFRGGLDV	HSYDSSIRGGI	+
60	HC group 141, LC group 162:	PWC2057	SRYSSSPFRGGLDV	HSYDSSISGGI	+
61	HC group 141, LC group 164:	PWC2093	SRYSSSPFRGGLDV	HSYDSSISAWI	+
62	HC group 141, LC group 170:	PWC2375	SRYSSSPFRGGLDV	HSYDSSISGWI	+
63	HC group 141, LC group 178:	PWC2652	SRYSSSPFRGGLDV	HSYDSSISGWI	+
64	HC group 141, LC group 187:	PWC2939	SRYSSSPFRGGLDV	HSYDSSISGWI	+
65	HC group 142, LC group 160:	PWC2068	SSSGCLFIDY	QSYDSSLGGYVI	+
66	HC group 143, LC group 157:	PWC2131	SRYSSSPFRGGLDV	HSYDSSISGWI	+
67	HC group 143, LC group 158:	PWC2892	SRYSSSPFRGGLDV	HSYDSSISGWI	++
68	HC group 144, LC group 155:	PWC2151	DSSSGWFFIDY	QSYDSSLGGYVI	+
69	HC group 145, LC group 157:	PWC2156	SRYSSSPFRGGLDV	HSYDSSISGWI	+
70	HC group 146, LC group 157:	PWC2321	SRYSSSPFRGGLDV	HSYDSSISGWI	+
71	HC group 147, LC group 157:	PWC2332	SRYSSSPFRGGLDV	HSYDSSISGWI	+
72	HC group 148, LC group 168:	PWC2350	DSSSGWFFI	QSYDSSLGGYVI	+
73	HC group 149, LC group 155:	PWC2386	SSSGWLFIDY	QSYDSSLGGYVI	+
74	HC group 150, LC group 171:	PWC2393	SRYSSSPFRGGLDV	HSYDSSISGWI	+
75	HC group 151, LC group 157:	PWC2412	SRYSSSPFRGGLDV	HSYDSSISGWI	+
76	HC group 152, LC group 155:	PWC2424	DSSSGWFFIDY	QSYDSSLGGYVI	+

77	HC group 153, LC group 155:	PWC2431	DSSSGWFFIDY	QSYDSSLGGYVI	+
78	HC group 154, LC group 157:	PWC2436	SRYSSSPFRGGLDV	HSYDSSISGWI	+
79	HC group 155, LC group 155:	PWC2444	DSSSGWFFIDY	QSYDSSLGGYVI	+
80	HC group 156, LC group 173:	PWC2590	TYPYGGGTYAFDY	QSYDSELSGSEL	-
81	HC group 157, LC group 174:	PWC2606	NAFDY	NSLDSRGQRVI	+
82	HC group 158, LC group 177:	PWC2643	SAKSGWKSTFDV	ALYLGGGLSWV	-
83	HC group 159, LC group 180:	PWC2682	No seq.	AAWDDSLSAYV	-
84	HC group 160, LC group 181:	PWC2691	DSSSGWLFIDY	QSYDSSLGGYVI	+
85	HC group 161, LC group 157:	PWC2710	SRYSSSPFRGGLDV	HSYDSSISGWI	+
86	HC group 161, LC group 182:	PWC2722	SRYSSSPFRGGLDV	HSYDSSISGWI	+
87	HC group 162, LC group 184:	PWC2758	DSSSGWLFIDY	QSYDSSLGGYVI	+
88	HC group 163, LC group 185:	PWC2771	DSSSGWLFIDY	QSYDSSLGGYVI	+
89	HC group 164, LC group 157:	PWC2792	SRYSSSPFRGGLDV	HSYDSSISGWI	+
90	HC group 165, LC group 186:	PWC2901	QMIMAARC	QSFDNRLRGFVV	-
91	HC group 166, LC group 155:	PWC2972	DSSSGWFFI	QSYDSSLGGYVI	+
92	HC group 167, LC group 155:	PWC2980	DSSSGWFFI	QSYDSSLGGYVI	+
93	HC group 148 LC group 138	PWD0103	VDHKWDLPFDY	ATWDDNLNGWV	+
94	HC group 148, LC group 194:	PWD0332	VDHKWDLPFDY	ATWDDSLNGWV	+
95	HC group 148, LC group 249:	PWD0853	VDHKWDLPFDY	ATWDDSLNGWV	+
96	HC group 148, LC group 272:	PWD1070	VDHKWDLPFDY	AAWDDSLNGWV	+
97	HC group 149, LC group 139:	PWD0104	LLRGGSTYLDAFDN	QVWDRSNGHVV	+
98	HC group 149, LC group 199:	PWD0384	LLRGGSTYLDAFDN	QVWDRSNGHVV	+
99	HC group 150, LC group 140:	PWD0106	GWGVFDI	AAWDDSLDGVV	+
100	HC group 151, LC group 141:	PWD0108	VDHNWDLPFDY	SAWDDSLNGWV	+
101	HC group 151, LC group 143:	PWD0111	VDHNWDLPFDY	ASWDDDLNGWV	+
102	HC group 151, LC group 195:	PWD0336	VDHNWDLPFDY	AVWDDRMNGWE	+
103	HC group 151, LC group 208:	PWD0435	VDHNWDLPFDY	AAWDDSLNGWV	+
104	HC group 151, LC group 234:	PWD0757	VDHNWDLPFDY	AVWDDRLNGWE	+
105	HC group 151, LC group 248:	PWD0848	VDHNWDLPFDY	VDHNWDLPFD	+
106	HC group 151, LC group 253:	PWD0875	VDHNWDLPFDY	AAWDDSLSGWM	+
107	HC group 151, LC group 257:	PWD0925	VDHNWDLPFDY	ASWDDDLKSWV	+
108	HC group 151,	PWD1048	VDHNWDLPFDY	AAWDDSLSGWV	+

	LC group 269:				
109	HC group 152, LC group 142:	PWD0109	VDHNWDLPFDY	ATWDDSLKGWV	+
110	HC group 152, LC group 164:	PWD0171	VDHNWDLPFDY	QQSKSIPIT	-
111	HC group 152, LC group 197:	PWD0366	VDHNWDLPFDY	VAWDDSLNGWM	+
112	HC group 152, LC group 214:	PWD0470	VDHNWDLPFDY	AAWDDSLSGWV	+
113	HC group 153, LC group 144:	PWD0112	VDHKWDLPFDY	AAWDDSLKGWV	+
114	HC group 153, LC group 227:	PWD0679	VDHKWDLPFDY	SAWDDGLSGWV	+
115	HC group 154, LC group 145:	PWD0114	VDHKWDLPFDY	ATWDDSLPGLV	+
116	HC group 154, LC group 215:	PWD0526	VDHKWDLPFDY	EAWDDSLSGPA	+
117	HC group 154, LC group 239:	PWD0810	VDHKWDLPFDY	AAWDDNLSGP	-
118	HC group 154, LC group 267:	PWD1012	VDHKWDLPFDY	QQTYRTPIT	+
119	HC group 154, LC group 270:	PWD1050	VDHKWDLPFDY	GTWDSRLYVGQV	+
120	HC group 155, LC group 146:	PWD0118	VDHNWDLPFDY	AAWDDSLNGWV	+
121	HC group 155, LC group 172:	PWD0205	VDHNWDLPFDY	AAWDDSLNGWV	+
122	HC group 155, LC group 241:	PWD0813	VDHNWDLPFDY	ATWDDSLNHWV	+
123	HC group 155, LC group 244:	PWD0827	VDHNWDLPFDY	AAWDDSLNGHW V	+
124	HC group 155, LC group 271:	PWD1063	VDHNWDLPFDY	AAWDDSLSGVL	+
125	HC group 156, LC group 147:	PWD0121	YVADTSKDVFDI	NSRDSSGNVV	+
126	HC group 157, LC group 148:	PWD0123	VASTALYFDN	ASWDDTLKGGV	+
127	HC group 158, LC group 149:	PWD0124	GVYNWNSAAKFDY	QSYDNSLSGSE	+
128	HC group 159, LC group 150:	PWD0127	TYYYVYYNYMDV	NSRDSSGDPVT	+
129	HC group 160, LC group 151:	PWD0130	VAHGWHLSFDY	SAWDDSLKGWV	+
130	HC group 161, LC group 152:	PWD0133	SLFRVRGVFFDY	ASRDSSANQHWV	+
131	HC group 161, LC group 158:	PWD0150	SLFRVRGVFFDY	QSYDSSTGI	+
132	HC group 162, LC group 153:	PWD0135	GPAGLQLSLDI	AAWDDSLNGLV	+
133	HC group 163, LC group 154:	PWD0136	VDHRWDLPFDY	STWDGSLNGWV	+
134	HC group 164, LC group 155:	PWD0139	VDHKWDLPFDY	AAWDDSLNGWV	+
135	HC group 164, LC group 163:	PWD0169	VDHKWDLPFDY	STWDDSLRGVV	+
136	HC group 164, LC group 181:	PWD0254	VDHKWDLPFDY	AVWDDSLNGWV	+
137	HC group 164, LC group 240:	PWD0811	VDHKWDLPFDY	APWDDSLNGWV	+
138	HC group 165, LC group 156:	PWD0146	ARDYYFGMDV	SAWDDSLHGPV	+
139	HC group 166, LC group 157:	PWD0147	GPAGLQLSLDI	AAWDDSLNGVV	+
140	HC group 167,	PWD0151	DRSKLNAGYFDS	QSYDNSLSAW	+

	LC group 159:				
141	HC group 168, LC group 160:	PWD0154	TKYSSIVFDL	AAWDDSLNVVV	+
142	HC group 169, LC group 161:	PWD0157	FRFLVWYGEAYFDY	SSRDNSGDRLVL	+
143	HC group 170, LC group 162:	PWD0164	VRGQLLAFDI	AAWDDSLNGWV	+
144	HC group 171, LC group 165:	PWD0175	VDHKWDLPFDY	ATWDDSLRGWV	+
145	HC group 171, LC group 182:	PWD0258	VDHKWDLPFDY	ATWDDSVRGWV	+
146	HC group 172, LC group 166:	PWD0176	GPAGLQLSLDI	ATWDDSLSGWV	+/-
147	HC group 173, LC group 167:	PWD0177	TKYSSIVFDL	AAWDDSLNAVL	+
148	HC group 174, LC group 168:	PWD0183	AVWDDSLNGH	VDRRWDLPFDY	+
149	HC group 175, LC group 169:	PWD0187	TKYSSIVFDL	ASWDDSLNGV	+
150	HC group 176, LC group 170:	PWD0189	LDHKWDLPFDY	EAWDDSLSGPA	+/-
151	HC group 177, LC group 171:	PWD0190	VDHNWDLPFDY	GTWDSRLSAVV	+
152	HC group 178, LC group 173:	PWD0211	EYYYRWGSYAN	NSRDSSGNPVV	+/-
153	HC group 179, LC group 174:	PWD0218	VDHKWDLPFDY	TAWDDSLNGWV	+
154	HC group 180, LC group 175:	PWD0228	VDHNWDLPFDY	AAWDDILNGWV	+
155	HC group 181, LC group 176:	PWD0229	SLFRVRGVFFDY	NSRDSSGNHVV	+
156	HC group 181, LC group 193:	PWD0329	SLFRVRGVFFDY	QAWDSSTTWE	+
157	HC group 181, LC group 233:	PWD0754	SLFRVRGVFFDY	ETWDTSLSVLV	+
158	HC group 182, LC group 177:	PWD0233	DLGVGRYFDY	SSRDNSGDPL	+
159	HC group 182, LC group 223:	PWD0611	DLGVGRYFDY	SSRDNSGDPL	+
160	HC group 183, LC group 178:	PWD0243	SLFRVRGVFFDY	NSRDSSGNHWV	+
161	HC group 184, LC group 179:	PWD0246	DRSKLNAGYFDS	QSYDSSLSAYV	+
162	HC group 184, LC group 263:	PWD0968	DRSKLNAGYFDS	QSYDSGLSAVV	+
163	HC group 185, LC group 180:	PWD0248	LDHNWDLPFDY	ASWDDSLSGWV	+
164	HC group 185, LC group 207:	PWD0427	LDHNWDLPFDY	ATWDDSLSGLL	+
165	HC group 185, LC group 235:	PWD0766	LDHNWDLPFDY	ASWDDSLKGVV	+
166	HC group 186, LC group 183:	PWD0259	TKYSSIVFDL	AAWDDRLSGPV	+
167	HC group 186, LC group 210:	PWD0441	TKYSSIVFDL	AAWDDSLNGML	+
168	HC group 186, LC group 243:	PWD0824	TKYSSIVFDL	AAWDDSLNGP	+/-
169	HC group 187, LC group 184:	PWD0268	LDHNWNLPFD	ATWDDRLKGFV	+
170	HC group 188, LC group 185:	PWD0283	VGGAIRFDS	SAWDDSLSGVV	+
171	HC group 189, LC group 186:	PWD0288	SVGRSLAFDI	AAWDDSLNGHVV	+
172	HC group 190,	PWD0291	RTGDCSYTSCY	QTWDSTTAS	+

	LC group 187:				
173	HC group 191, LC group 188:	PWD0294	GPAGLQLSLDI	SAWDDSLNGPA	+
174	HC group 191, LC group 209:	PWD0440	GPAGLQLSLDI	SAWDDSLNGPA	+
175	HC group 192, LC group 189:	PWD0305	VDHKWDLPFDY	ATWDDTLSGLV	+
176	HC group 193, LC group 190:	PWD0308	FTGWYGAFDI	ATWDDSVNGPA	+
177	HC group 194, LC group 191:	PWD0318	DRYNMVGVLRPDS	SSYARSNNFGV	-
178	HC group 195, LC group 192:	PWD0323	QIWGRFEY	AAWDDRLNGYV	+
179	HC group 195, LC group 219:	PWD0587	QIWGRFEY	AAWDDSLNGVV	+
180	HC group 196, LC group 196:	PWD0339	GYDFWSGFDY	QVWDSTSDHRI	+
181	HC group 197, LC group 140:	PWD0355	GWGVFDM	AAWDDSLDGVV	+
182	HC group 198, LC group 198:	PWD0369	VDHKWDLPFDF	ASWDDSLDGWV	+/-
183	HC group 199, LC group 200:	PWD0389	ARALFRVSGPY	SSYSGDVNFIV	+
184	HC group 200, LC group 201:	PWD0391	DHPYNWNYFDY	QQLNRYPSL	-
185	HC group 201, LC group 202:	PWD0392	GAPAVRHGFDY	QQYYSTPPT	-
186	HC group 202, LC group 203:	PWD0412	VDHKWDLPFDY	ATWDDSLKGFV	+
187	HC group 203, LC group 204:	PWD0416	FGTGSSLEV	AAWDDSLNGVV	+
188	HC group 204, LC group 205:	PWD0422	QAFARFEF	SSWDDSLNGVV	+
189	HC group 205, LC group 206:	PWD0424	NLQDIVATILPFDY	GTWDSSLNTYV	-
190	HC group 206, LC group 141:	PWD0436	VDHNWDLPFDY	SAWDDSLNGWV	+
191	HC group 207, LC group 211:	PWD0451	GDPEELRSDSYFYYGMDV	QSYDSSLSGSWV	-
192	HC group 208, LC group 212:	PWD0454	LDHKWDLPFDH	EAWDDSLSGPA	+
193	HC group 209, LC group 213:	PWD0469	TKYSSVAFDL	ATWDDSLNGVV	+
194	HC group 210, LC group 139:	PWD0525	LLRGGSTYLDAFDX	QVWDRSNGHVV	+
195	HC group 211, LC group 216:	PWD0541	Not Determined	QQFKSYPLT	-
196	HC group 212, LC group 217:	PWD0542	GVYGGGSAGLYFDV	QVWDNSSGWV	+
197	HC group 213, LC group 218:	PWD0568	GEMATIRY	ATWDDSLNGWV	+
198	HC group 214, LC group 220:	PWD0588	VDHKWDLPFDY	AAWDASLTSWV	+
199	HC group 215, LC group 221:	PWD0593	VDHNWDLPFDY	AAWDDSLNGVV	+
200	HC group 216, LC group 222:	PWD0605	ASSWYLVFDI	AAWDDSLNGWV	+
201	HC group 216, LC group 259:	PWD0929	ASSWYLVFDI	AAWDDSLNGWV	+
202	HC group 217, LC group 224:	PWD0615	SLFRVRGVFFDY	GTWDSSLSDGKVV	+

203	HC group 218, LC group 146:	PWD0635	VDHNWDLPFDY	AAWDDSLNGWV	+
204	HC group 219, LC group 225:	PWD0638	VDHNWDLPFDY	ATWDDSRGGWV	+
205	HC group 220, LC group 226:	PWD0648	VDRRWDLPFDY	ASWDDSVGSWV	+
206	HC group 221, LC group 228:	PWD0706	VDHKWDLPFDF	AAWDDSLNGWV	+
207	HC group 222, LC group 229:	PWD0709	GGPPFGSSYDV	ASWDDDLSGLV	+
208	HC group 223, LC group 230:	PWD0718	PTYGPGSFLIDH	ATWDDSLNGPV	+
209	HC group 224, LC group 231:	PWD0721	TRGYSLYFDS	ATWDDSLMVGV	+
210	HC group 225, LC group 232:	PWD0730	VDHNWDLPFDY	ATWDDSLNGWV	+
211	HC group 226, LC group 236:	PWD0773	GPAGLQLSLDI	AVWDDSLNGVI	+
212	HC group 227, LC group 237:	PWD0776	GPAGLQLSLDI	AAWDDNLNGVV	+/-
213	HC group 228, LC group 144:	PWD0791	VDHKWDLPFDY	AAWDDSLKGWV	+
214	HC group 229, LC group 238:	PWD0808	AGGSSLVFDS	AVWDDGLSGWV	+
215	HC group 230, LC group 242:	PWD0821	DGPSNYMDV	QQYYSTPIT	-
216	HC group 231, LC group 245:	PWD0830	VDHNWDLPFDY	VAWDDSLNGWV	+
217	HC group 232, LC group 246:	PWD0834	DGDYSSSSLDY	QSHDNTLGEV	-
218	HC group 233, LC group 247:	PWD0838	VRVPGRDGMDV	ASWDDSLTWV	-/+
219	HC group 234, LC group 250:	PWD0858	GSGSYIAFDI	AAWDDSLSGPVV	+
220	HC group 235, LC group 251:	PWD0864	TTVTTESDWFDL	NSRDSSGNHFDVV	-/+
221	HC group 236, LC group 252:	PWD0871	VDHNWDLPFDY	ATWDDSLNGFV	+
222	HC group 237, LC group 217:	PWD0876	GVYGGGSAGLYFDV	QVWDNSSGWV	+
223	HC group 238, LC group 254:	PWD0880	GPSGLLLGLDV	AVWDDSLNGVL	+
224	HC group 239, LC group 255:	PWD0884	VASTALYFDN	AAWDDSLTGWV	+
225	HC group 240, LC group 256:	PWD0914	LSGVTLHMDV	AAWDDSLKGPV	+
226	HC group 241, LC group 258:	PWD0928	VRGGNLAFDF	AAWDDSLSGWV	+
227	HC group 242, LC group 260:	PWD0934	SLFRVRGVFFDY	VTWDGSLGVVM	+
228	HC group 243, LC group 144:	PWD0948	EDHKWDLPFDY	AAWDDSLKGWV	+
229	HC group 244, LC group 261:	PWD0949	GALSSFDS	AAWDDSLNGWV	+
230	HC group 245, LC group 262:	PWD0953	QIWGRFEY	AAWDDSLNGVV	+
231	HC group 246, LC group 146:	PWD0963	ADHNWDLPFDY	AAWDDSLNGWV	+
232	HC group 247 LC group 264:	PWD0991	AHWGSRVDY	AAWDDSLNGVV	+
233	HC group 248, LC group 265:	PWD0995	LLRGGSTYLDAFDN	QVWDRSNGHVV	+
234	HC group 249,	PWD1003	Not Determined	NSRDSSGNLWV	-

	LC group 266:				
235	HC group 250, LC group 268:	PWD1038	EVGSYFDY	AAWDDSLNGVV	+
236	HC group 251, LC group 273:	PWD1072	VDHNWDLPFDY	AAWDDSLNGWV	+
237	HC group 252, LC group 274:	PWD1077	SLFRVRGVFFDY	NSRDNSGNLWV	+
238	HC group 253, LC group 275:	PWD1079	GPRFWTGYYDY	QQSLTAWT	+

Example 2: Affinity Ranking of mAbs to PA and PA Cleavage Site Peptide ELISA

[0347] Theoretical considerations suggest that under ideal circumstances antibody concentration at half-maximal antigen binding (EC50) is a measure of affinity. In practical terms it can be used to rank the affinities of antibodies to quickly identify best binders. The lower the antibody concentration required for 50% of plateau binding, the higher is the affinity of the antibody for antigen. In the approach described below, a conventional ELISA is used to generate binding isotherms for PA antibodies in order to derive their EC-50 values. Additionally, antibodies may be tested for their ability to bind peptides that span the RKKR cleavage site in PA.

EC-50 ELISA

[0348] Direct Plate Coating with PA: 50 microliters of PA solution (0.2 μg/ml in PBS) is dispensed to individual wells of 96- well plates (Immulon-2, Dynex) sealed with Plate sealers (Advanced Genetic cat.# 48461) and incubated overnight at 4°C. Next day the coating solution is removed, plates are washed 4 times with PBS with 0.1% Tween-20 and blocked by incubation with 200 microliters of blocking buffer (PBS, 3%BSA) for 1 hr at room temperature.

[0349] Serial dilutions of anti-PA antibodies are prepared in diluent buffer (PBS, 0.1% Tween-20, 0.1% BSA). Human IgG2 (Sigma, cat# I-4139) is used as a negative control. Two 50 microliter aliquots of each dilution are dispensed into individual wells of coated and blocked plates. The plates are sealed and incubated for 2 hours at room temperature.

[0350] Next, plates are washed 4 times with PBST (PBS, 0.1% Tween-20) and 50 microliters of HRP labeled anti-human IgG (Vector, cat# PI-3000) at concentration 1 microgram/milliliter in diluent buffer is dispensed to individual wells. Plates are sealed and incubated for 1 hr at room temperature. In the meantime substrate solution is prepared by dissolving 1 tablet of TMB (Sigma cat# T3405) in 5 ml of water. After the tablet is

dissolved, 5 ml of the substrate buffer (0.1 M Na_2PO_4, 0.05 M Citric acid) and 2 microliters of 30% H_20_2 is added.

[0351] Plates are washed 4 times with PBST and 100 microliters of substrate is added to each well. Plates are incubated for 10 min at room temperature and the Absorption at 450 nm is measured on SpectraMax 3000 (Molecular Devices).

[0352] Data analysis Data is analyzed using SofMaxPro 3.0. Binding curves (on OD 450 versus concentration graphs) are generated using the four parameter fit model. EC-50 values are calculated automatically as the concentration of the antibody that provides 50% of the maximum binding (maximum binding is characterized by parameter D in the four parameter fit equation).

Cleavage site peptide ELISA:

[0353] Indirect coating of biotinylated peptides to streptavidin coated plates: One hundred microliters of Streptavidin (Sigma S-4762) solution (1 mg/ml in PBS) is dispensed into individual wells of 96-well plates (Immulon-4, Dynex) sealed with Plate sealers (Advanced Genetic Cat.# 48461) and incubated overnight at 4°C. The next day the coating solution is removed, plates are washed 4 times with PBS + 0.1% Tween-20 and blocked by incubation with 200 ml of blocking buffer (PBS, 3% BSA) for 1 hour at room temperature.

[0354] After blocking, the blocking solution is removed, plates are washed 4 times with PBS + 0.1% Tween-20 and 100 microliters per well of biotinylated peptides (1 mg/ml diluted in 0.1% BSA in PBS) is incubated for 1 hour at room temperature. The biotinylated peptides are: sp-186: biotin-SNSRKKRSTSAGPTVPDRDN (amino acids 190-206 of SEQ ID NO:2); sp-187: biotin-QLPELKQKSSNSRKKRSTSAG (amino acids 181-201 of SEQ ID NO:2); and sp-189: biotin-QLPELKQKSSNSRKK (amino acids 181-195 of SEQ ID NO:2). The plates are then washed 4 times with PBST.

[0355] 100 microliters of 3 dilutions (10 micrograms/ml, 1.0 micrograms/ml and 0.1 micrograms/ml) of purified antibody in duplicate are dispensed into the 96-well plate. The plates are sealed and incubated for 2 hours at room temperature. Plates are washed 4 times with PBST and 100 microliters of HRP-labeled goat anti human IgG (H+L) (Vector, Cat# PI-3000) are dispensed into individual wells (1 mg/ml in 0.1% BSA in PBST). Plates are sealed and incubated for 1 hour at room temperature. In the meantime, substrate solution is prepared by dissolving 1 tablet of TMB (Sigma, Cat# T3405) in 5 ml of water.

After the tablet is dissolved, 5 ml of the substrate buffer (0.1 M Na_2PO_4, 0.05 M Citric acid) and 2 ml of 30% H_2O_2 are added. Plates are washed 4 times with PBST and 100 microliters of substrate is added to each well. Plates are incubated for 15 minutes at room temperature and the absorption (at 450 nm) is measured on SpectraMaxPlus (Molecular Devices).

Example 3: Inhibition of Biotinylated PA Binding to Anthrax Receptors

[0356] The following protocol may be used to test whether an antibody is able to inhibit the binding of biotinylated PA protein to the anthrax receptor protein (SEQ ID NO:3).

Preparation of ATR protein

[0357] ATR protein was produced by cloning the first 227 amino acid residues of the ATR protein gene (SEQ ID NO:3, Bradley et al, (2001) *Nature* 414:225-229) linked to a polynucleotide encoding the FLAG® tag (Stratagene, La Jolla, CA) (amino acid residues DYKDDDDK, SEQ ID NO:43) into a mammalian expression vector (Lonza Biologics). Recombinant soluble ATR protein was expressed from 293T cells by transient transfection. Three days after transfection, conditioned media were collected and the ATR protein with a flag-tag at the carboxy terminus was purified from the media sample by passing it through an anti-flag monoclonal antibody affinity column (Sigma).

Preparation of PA protein

[0358] PA protein was produced from a synthetic gene encoding the *B. anthracis* PA which was constructed using a combination of overlapping oligonucleotides and polymerase chain reaction (PCR). The synthetic gene encoding the *B. anthracis* PA residues E30-G764 of mature PA (SEQ ID NO:2) was codon-optimized for expression in the bacterium, *Escherichia coli*. Subsequent PCR amplification reactions were performed to add a heterologous signal peptide to the N-terminus of mature PA (E30-G764). PA was produced in *E. coli* K-12 cells and extracted from the cell paste upon periplasmic shock. The PA protein was purified using Q-sepharose-HP and hydroxyapatite chromatography. PA purity was confirmed by native- and SDS-PAGE silver staining, N-terminal protein sequencing, RP-HPLC and SEC-HPLC and was determined to be 96-98% pure.

[0359] To assess the proper function of the PA protein, the ability of the PA protein to form heptamers after enzymatic cleavage was evaluated. PA binds to its cell surface receptor and is cleaved by a furin-like protease to eliminate the N-terminal 20 kilodalton region. This cleavage permits the PA63 fragment to polymerize and form a heptameric pore in the membrane. PA63 can also be obtained in vitro by trypsin digestion (Benson et al, (1998) *Biochemistry* 37:3941-3948; Ahuja et al, (2001) *Biochem. Biophys. Res. Comm.* 286:6-11, each of which are herein incorporated by reference in their entireties). Purified PA (83 kilodaltons) was subjected to trypsin digestion to determine if the purified PA was capable of being cleaved to PA63 and if the cleaved PA63 fragments could form heptamers. The trypsin-treated PA protein was analyzed via SDS-PAGE and transferred to a membrane for protein sequencing. SDS-PAGE analysis indicated the trypsin-treated PA protein yielded a 63 kilodalton protein. Subsequent N-terminal sequence analysis confirmed the correct cleavage position. Moreover, native-PAGE analysis and mass spectrometry indicated that the 63 kilodalton subunits formed multimers. Heptameric PA63 was also captured on a Q-sepharose-HP column.

Inhibition of PA binding to ATR Assay

[0360] The assay buffer for this assay consists of 1xPBS (pH7.4, without calcium and Magnesium; catalogue # 17-516 from BioWhittaker), 2% BSA (Sigma, A-0336, stock 30% solution), 1mM $CaCl_2$, 1mM $MgCl_2$, 0.1% Tween-20. Calcium and magnesium need to be added to assay buffer at the time of assay. Biotinylated PA protein (final concentration 300 ng/ml; biotinylation was performed using EZ-Link™ Sulfo-NHS-LC-Biotin available from Pierce Biotechnology) is pre-incubated with antibody preparations (phage expressing scFv, purified scFv or whole antibody molecules, such as IgG molecules, comprising the VH and VL domains of specific scFvs) in assay buffer for 45 minutes, at room temperature with gentle shaking. Flag-tagged ATR protein (amino acids 1- 227 of SEQ ID NO:3) is then added to the mixture and incubated for an additional 20 minutes.

[0361] Next, 2.5 microliters of streptavidin coated beads (Dynabeads M-280, Dynal Biotech) is added to each well along with anti-flag antibody (Sigma, catalogue # F3165) (1 microgram/milliliter final concentration) that has been labeled with ORI-TAG® (IGEN International) according to the manufacturer's directions. The mixture is then incubated

for 45 minutes at room temperature with gentle shaking. Electochemiluminescence is then measured using the M8 ECL unit (IGEN, International).

[0362] The protein concentrations given in the above-described assay can be modified by one of skill in the art to optimize assay performance, as necessary. Additionally, this assay may be modified to test an antibody's ability to block binding of PA to its receptor on other primary cells or cell lines, such as macrophage cell lines.

[0363] Figure 1 shows results for the ability of antibodies PWD0283 and PWD0587 to inhibit the binding of biotinylated PA to ATR.

Inhibition of PA binding to CMG2

[0364] In an assay similar to the one described above antibody PWD0587 was tested for its ability to block binding of PA to a flag tagged version of CMG2 protein that also acts as an anthrax receptor (see, Scobie et al., (2003) *Proceedings of the National Academy of Sciences* 100:5170-5174). The CMG2 protein used consisted of amino acids 33-318 of SEQ ID NO:42 fused to a flag tag (SEQ ID NO:43). Using this assay, it was shown that an IgG1 format of the PWD0587 antibody also inhibits the binding of PA to CMG2.

Example 4: Detection of Biotinylated PA binding to PA Receptor by Flow Cytometry

[0365] In preparation for a series of in vitro studies to test if anti-PA monoclonal antibodies of the invention can inhibit the action of PA, flow cytometry analysis of binding of biotinylated PA protein to CHO-K1 cells, J774A.1 cells, and human macrophages was performed. CHO-K1 cells are a cell line that have functional ATR protein on their surface. Both the J774A.1 cells (a murine macrophage cell line) and human macrophages also possess PA binding proteins on their surface. For flow cytometry analysis, PA protein was biotinylated as described above (Example 3) and added to cells in culture and incubated for 10-20 minutes at room temperature. The cells were washed and pelleted by centrifugation. Streptavidin PE was then added to the cell pellets and incubated briefly at room temperature. After washing, the cells were first resuspended in propidium iodide to discriminate between live and dead cells, and then were analyzed on a FACScan. Data were acquired and analyzed using CellQuest software (Becton Dickinson). As shown in Figure 2, biotinylated PA binds specifically to CHO-K1

cells, J774A.1 murine macrophages, and human macrophages, indicating that these cells have ATR protein on their surface.

Example 5: ^{86}Rubidium Release Assay

[0366] PA has been shown to interact with the ATR on CHO-K1 cells (Escuyer and Collier, (1991) *Infect. Immunol.* 59:3381-3386, which is hereby incorporated by reference in its entirety. Following binding of PA to the ATR, a 20 kilodalton peptide is cleaved from the PA and the remaining PA63 molecules aggregate into heptamers and form a pore on the cell surface. One assay that has been developed to measure PA63-mediated pore formation monitors the release of intracellular ^{86}Rubidium (^{86}Rb) from cells that have been pre-loaded with ^{86}Rb. The following protocol may be used to test whether an antibody is able to inhibit the ability of PA63 to form pores in membranes.

^{86}Rubidium Release Assay Using CHO-K1 cells

[0367] 2.0 x 10^5 CHO-K1 cells (ATCC#CCL 61, which express PA Receptor on their surface, see Example 4) are plated in a 24 well plate in 1 milliliter of culture medium (Ham's F12K medium with 2 mM L-glutamine adjusted to contain 2.5 g/L sodium bicarbonate., 90%; fetal bovine serum, 10%). The cells are incubated for 24 hours at 37°C, 5%CO_2. Medium is aspirated and replaced with milliliter of fresh culture medium containing ^{86}Rb at a concentration 1 microCurie/milliliter. Cells are incubated on ice for 30 minutes after which the medium is removed and the cells are washed two times with 750 µl of cold PBS. Next, 1 milliliter of medium containing PA alone or PA which has been pre-incubated (for 1 hour at 37 degrees on a rotator) with anti-PA antibody is added to the cells which are then incubated for 1.5 hours on ice. At the end of the incubation period, the cells are again washed twice with 750 µl of cold PBS. 500 microliters of cold MES-gluconate buffer, pH4.9 is then added and incubated on ice for 30 minutes. 100 microliters of supernatant is removed from cell supernatant and added to 2.0 ml of Supermix OptiPhase scintillant (Perkin-Elmer Life Sciences) and the radioactivity is counted using a Wallac Microbeta TRILUX Liquid Scintillation and Luminescence Counter (Perkin-Elmer). Radioactivity in the medium is indicative of pore formation by PA63.

[0368] To determine the optimal amount of PA to use in the ^{86}Rb release assay, PA was titrated by measuring ^{86}Rb release with different concentrations of PA (18, 6, 2,

0.67, 0.22, 0.074, 0.025, and 0 nM) were added to the assay. Based on this titration, 5 nM PA was chosen for the subsequent assays.

[0369] In this assay PATD, a mutant of PA which is defective in pore formation is used as a positive control for inhibition of pore formation. PATD is identical to wildtype PA with the exception that it has two amino acid mutations, K426D and D454K using the numbering of SEQ ID NO:2. PATD is described in Sellman et al., *J. Biol. Chem.* (2001), 276:8371-6, Sellman et al., *Science* 292:695-697, and in International Patent Publication WO01/82788 each of which is hereby incorporated by reference in its entirety.

[0370] Figure 3 shows the ability of two antibodies PWD0283 and PWD0587 in whole IgG1 format to inhibit pore formation by PA protein using the above described assay.

^{86}Rubidium Release Assay Using Human Macrophages

[0371] The systemic shock and death from anthrax results primarily from the effects of high levels of cytokines produced by and released from macrophages that have been affected by the anthrax lethal toxin. Consequently, it was of interest to evaluate whether anti-PA antibodies of the invention could inhibit PA-mediated release of ^{86}Rb from human macrophages.

Preparation of Human Macrophages

[0372] For the preparation of human macrophages, peripheral blood mononuclear cells (PBMC) were isolated from various human donors by Ficoll-Hypaque density gradient centrifugation. PBMC were incubated with anti-CD14-labeled paramagnetic microbeads (Miltenyi Biotec). After magnetic labeling, the cells were passed through a separation column placed in a strong permanent magnet. The magnetically-labeled cells retained in the column were then eluted, washed, and counted. CD14+ cells were then placed in 6-well culture dishes for 10-12 days in medium containing granulocyte-macrophage colony-stimulating factor (GM-CSF). Medium was replenished every 3 days until the cells were used in the assay.

Inhibition of ^{86}Rb Release from Human Macrophages by anti-PA Monoclonal Antibodies

[0373] The ^{86}Rb release assay was performed as described above, except for using human macrophages in place of the CHO-K1 cells. Anti-PA monoclonal antibodies PWD0283 and PWD0587 fully inhibited PA-mediated ^{86}Rb release at antibody concentration of about 5 nM.

Example 6: Identification and Cloning of VH and VL domains

[0374] One method to identify and clone VH and VL domains from cell lines expressing a particular antibody is to perform PCR with VH and VL specific primers on cDNA made from the antibody expressing cell lines. Briefly, RNA is isolated from the cell lines and used as a template for RT-PCR designed to amplify the VH and VL domains of the antibodies expressed by the EBV cell lines. Cells may lysed in the TRIzol® reagent (Life Technologies, Rockville. MD) and extracted with one fifth volume of chloroform. After addition of chloroform, the solution is allowed to incubate at room temperature for 10 minutes, and the centrifuged at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. The supernatant is collected and RNA is precipitated using an equal volume of isopropanol. Precipitated RNA is pelleted by centrifuging at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. Following centrifugation, the supernatant is discarded and washed with 75% ethanol. Following washing, the RNA is centrifuged again at 800 rpm for 5 minutes at 4°C. The supernatant is discarded and the pellet allowed to air dry. RNA is the dissolved in DEPC water and heated to 60°C for 10 minutes. Quantities of RNA can determined using optical density measurements.

[0375] cDNA may be synthesized, according to methods well-known in the art, from 1.5-2.5 micrograms of RNA using reverse transcriptase and random hexamer primers. cDNA is then used as a template for PCR amplification of VH and VL domains. Primers used to amplify VH and VL genes are shown in Table 6. Typically a PCR reaction makes use of a single 5' primer and a single 3' primer. Sometimes, when the amount of available RNA template is limiting, or for greater efficiency, groups of 5' and/or 3' primers may be used. For example, sometimes all five VH-5' primers and all JH3' primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0.7 units of High Fidelity Taq polymerase, 5' primer mix, 3' primer mix and 7.5 microliters of cDNA. The 5' and 3' primer mix of both VH and VL can be made by pooling together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are: 96°C for 5 minutes; followed by 25 cycles of

94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; followed by an extension cycle of 72°C for 10 minutes. After the reaction is completed, sample tubes were stored 4°C.

Table 5: Primer Sequences Used to Amplify VH and VL domains.

Primer name	SEQ ID NO	Primer Sequence (5'-3')
VH Primers		
Hu VH1-5'	6	CAGGTGCAGCTGGTGCAGTCTGG
Hu VH2-5'	7	CAGGTCAACTTAAGGGAGTCTGG
Hu VH3-5'	8	GAGGTGCAGCTGGTGGAGTCTGG
Hu VH4-5'	9	CAGGTGCAGCTGCAGGAGTCGGG
Hu VH5-5'	10	GAGGTGCAGCTGTTGCAGTCTGC
Hu VH6-5'	11	CAGGTACAGCTGCAGCAGTCAGG
Hu JH1,2-5'	12	TGAGGAGACGGTGACCAGGGTGCC
Hu JH3-5'	13	TGAAGAGACGGTGACCATTGTCCC
Hu JH4,5-5'	14	TGAGGAGACGGTGACCAGGGTTCC
Hu JH6-5'	15	TGAGGAGACGGTGACCGTGGTCCC
VL Primers		
Hu Vkappa1-5'	16	GACATCCAGATGACCCAGTCTCC
Hu Vkappa2a-5'	17	GATGTTGTGATGACTCAGTCTCC
Hu Vkappa2b-5'	18	GATATTGTGATGACTCAGTCTCC
Hu Vkappa3-5'	19	GAAATTGTGTTGACGCAGTCTCC
Hu Vkappa4-5'	20	GACATCGTGATGACCCAGTCTCC
Hu Vkappa5-5'	21	GAAACGACACTCACGCAGTCTCC
Hu Vkappa6-5'	22	GAAATTGTGCTGACTCAGTCTCC
Hu Vlambda1-5'	23	CAGTCTGTGTTGACGCAGCCGCC
Hu Vlambda2-5'	24	CAGTCTGCCCTGACTCAGCCTGC
Hu Vlambda3-5'	25	TCCTATGTGCTGACTCAGCCACC
Hu Vlambda3b-5'	26	TCTTCTGAGCTGACTCAGGACCC
Hu Vlambda4-5'	27	CACGTTATACTGACTCAACCGCC
Hu Vlambda5-5'	28	CAGGCTGTGCTCACTCAGCCGTC
Hu Vlambda6-5'	29	AATTTTATGCTGACTCAGCCCCA
Hu Jkappa1-3'	30	ACGTTTGATTTCCACCTTGGTCCC
Hu Jkappa2-3'	31	ACGTTTGATCTCCAGCTTGGTCCC
Hu Jkappa3-3'	32	ACGTTTGATATCCACTTTGGTCCC
Hu Jkappa4-3'	33	ACGTTTGATCTCCACCTTGGTCCC
Hu Jkappa5-3'	34	ACGTTTAATCTCCAGTCGTGTCCC
Hu Jlambda1-3'	35	CAGTCTGTGTTGACGCAGCCGCC
Hu Jlambda2-3'	36	CAGTCTGCCCTGACTCAGCCTGC
Hu Jlambda3--3'	37	TCCTATGTGCTGACTCAGCCACC
Hu Jlambda3b-3'	38	TCTTCTGAGCTGACTCAGGACCC
Hu Jlambda4-3'	39	CACGTTATACTGACTCAACCGCC
Hu Jlambda5-3'	40	CAGGCTGTGCTCACTCAGCCGTC
Hu Jlambda6-3'	41	AATTTTATGCTGACTCAGCCCCA

[0376] PCR samples are then electrophoresed on a 1.3% agarose gel. DNA bands of the expected sizes (~506 base pairs for VH domains, and 344 base pairs for VL domains) can be cut out of the gel and purified using methods well known in the art. Purified PCR products can be ligated into a PCR cloning vector (TA vector from Invitrogen Inc., Carlsbad, CA). Individual cloned PCR products can be isolated after transfection of *E.*

coli and blue/white color selection. Cloned PCR products may then be sequenced using methods commonly known in the art.

Example 7: Kinetics of PA binding analyzed by BIACORE

[0377] For BIAcore analysis, PA and PA heptamer were immobilized on individual flow cells of a BIAcore CM5 sensor chip. The PA monoclonal antibodies, PWD0283 and PWD0587 (IgG1 format), were diluted from 50 μg/mL (333 nM) to 0.625 μg/mL (4.1 nM). Each concentration was in contact with the PA proteins during a 4-minute association phase. The off-rate of the anti-PA monoclonal antibodies was determined by washing the complex in the presence of buffer for 5 minutes. The binding data were analyzed using the BIAevaluation software, Version 3.1. The kinetics of anti-PA monoclonal antibody binding to PA and to PA heptamer are summarized in Tables 6 and 7, respectively. Both PWD0283 and PWD0587 antibodies showed high affinity binding to both PA and its heptamer.

Table 6 Kinetics of anti-PA monoclonal antibody binding to PA

Anti-PA mAb	ka (1/Ms)	kd (1/s)	KD (M)
PWD0283	4.46×10^{6}	1.03×10^{-3}	2.32×10^{-10}
PWD0587	2.44×10^{5}	5.30×10^{-4}	2.17×10^{-9}

ka (1/Ms, association rate constant; kd (1/s), dissociation rate constant; KD (M)

Table 7 Kinetics of anti-PA monoclonal antibody binding to PA heptamer

Anti-PA mAb	ka (1/Ms)	kd (1/s)	KD (M)
PWD0283	2.28×10^{6}	4.26×10^{-4}	1.87×10^{-10}
PWD0587	3.23×10^{5}	6.50×10^{-5}	2.01×10^{-10}

ka (1/Ms, association rate constant; kd (1/s), dissociation rate constant; KD (M)

Example 8: Inhibition of Lethal Toxin Mediated Cell Killing by anti-PA Antibodies

[0378] The ability of anti-PA antibodies to inhibit cell killing caused by lethal toxin (PA/LF) was evaluated using J774A.1, murine macrophage cell line (Quinn et al, (1991) *J*

Biol. Chem. 266:20124-20130, herein incorporated by reference in its entirety). The cells were seeded in a 96-well micro titer plate and incubated overnight. The next day, fresh medium containing 100 ng/mL PA was added. Then, 20 μL of DMEM (containing 100 ng/mL PA) and 50 ng/mL LF was added. Cells were incubated for 3 hrs. To detect viable cells after lethal toxin treatment, 20 μL of CellTiter 96 AQ_{ueous} One Solution Reagent (Promega) was added to each well and cells were incubated for 2.5 hrs. Plates were then read at 490 nm using SpectraMax250 (Molecular Devices). CellTiter 96 AQ_{ueous} One Solution Reagent contains a tetrazolium compound which is bioreduced by metabolically active cells into a soluble colored formazan product. The quantity of formazan product as measured by absorbance at 490 nm is directly proportional to the number of living cells.

[0379] The ability of PA mAb, PWD0283 and PWD05687, to inhibit cell killing was compared with a negative control IgG1 mAb (CAT002). As shown in Figure 4, PWD0283 and PWD0587 both inhibited lethal toxin-induced cell killing in a dose-dependent manner.

Example 9: Prophylactic Use of Anti-PA Antibodies

[0380] Fisher 344 rats are highly susceptible to the lethal effects of systemic doses of lethal toxin (Sellman et al., (2001) *Science* 292:695-697 and Ivins et al., (1989) *Applied and Environmental Microbiology* 55:2098-2100, both of which are herein incorporated by reference in their entireties). Lethal toxin is the combination of the receptor-binding component, PA and the metalloprotease, LF, of *B. anthracis*. The following studies were performed to examine the ability of anti–PA antibodies to act prophylactically by intravenous (IV), subcutaneous (SC) or intramuscular (IM) administration when administered at various times before single or multiple injections of lethal toxin (also referred to as "PA/LF" in this example and Example 10). In these studies, the time to morbundity (TTM) was measured and the number of animals surviving at 24 hours were counted. The average TTM following injection of lethal toxin is approximately 90 minutes. Animals that survived past 24 hours were euthanized.

[0381] PA (83 kilodaltons) was formulated at a concentration of 0.45 mg/mL in a buffer containing 50 mM $NaPO_4$ and then diluted with phosphate-buffered normal saline to concentrations of 0.1125 mg/mL and 0.2 mg/mL. A volume of 0.2 mL delivered 0.0225 mg or 0.04 mg of PA. Doses of 0.09 mg/kg or 0.16 mg/kg were used. The dose 0.09 mg/kg was based on the lowest concentration needed to produce 100% lethality. The

doses of PA monoclonal antibody and control monoclonal antibody used were in 10-fold molar excess of the PA dose.

[0382] Recombinant lethal factor (LF) from *B. anthracis*; List Biological Laboratories, Inc. (408.866.6363); Lot 1721B was provided as a lyophilized powder. When reconstituted with 1 mL sterile water for injection, the solution contained 1.0 mg LF in a buffer of 5 mM HEPES and 50 mM NaCl. It was then diluted with phosphate-buffered normal saline to a concentration of 0.040 mg/mL. A volume of 0.2 mL delivered 0.008 mg of LF. A dose of 0.032 mg/kg was used. This dose was based on the lowest concentration needed to produce 100% lethality.

Prophylactic Study 1: IV Administration of anti-PA Antibodies 60 Minutes Prior to Injection of Lethal Toxin

[0383] In this study, the effects of PA mAb administered 60 minutes prior to a single intravenous injection of PA/LF were examined. Male Fisher 344 rats (n = 5/treatment) were assigned to the groups shown in Table 8. Sixty minutes before intravenous injection of PA/LF, animals received either anti-PA monoclonal antibodies (PWD0283 or PWD0587 in IgG1 format), a negative IgG1 control monoclonal antibody (CAT002), vehicle, or no study agent by intravenous injection.

[0384] As shown in Table 8 and Figure 5, a single intravenous injection of PWD0283 or PWD0587 60 minutes prior to injection of lethal toxin provided 100% survival at 24 hours with no apparent ill effects. In contrast, a single injection of the negative control mAb, CAT002, provided no protection with 0% survival and an average TTM of 100 minutes. Vehicle or no study agent also provided no protection with 0% survival and an average TTM of 99 minutes and 91 minutes, respectively. In a separate study, rats receiving mAb alone without PA/LF showed no adverse effects.

Table 8

Group	n	Time of Study Agent relative to PA/LF (min)	Study Agent (3 mg/kg)	PA (mg/kg)	LF (mg/kg)	% Survival	TTM (minutes)
1	5	-60	PWD0283	0.16	0.032	100	-
2	5	-60	PWD0587	0.16	0.032	100	-
3	5	-60	CAT002	0.16	0.032	0	100
4	5	-60	vehicle	0.16	0.032	0	99
5	5	-	-	0.16	0.032	0	91

Prophylactic Study 2: SC and IM Administration of anti-PA Antibodies 60 Minutes Prior to Injection of Lethal Toxin

[0385] Because intravenous administration of anti-PA antibodies given 1 hour prior to lethal toxin was completely protective against lethal toxin, the experiment was repeated giving the anti-PA antibodies by SC or IM administration 60 minutes prior to the lethal toxin to examine these routes of PA mAb administration. Male Fisher 344 rats (n = 5/treatment) were assigned to the groups shown in Table 9. Sixty minutes before administration of PA/LF, animals received either PA mAb (PWD0283 or PWD0587), or a negative control antibody (CAT002).

[0386] SC administration of PWD0283 or PWD0587 (Table 9) 1 hour prior to lethal toxin administration provided no protection with 0% survival and an average TTM of 105 minutes and 145 minutes, respectively. IM administration of PWD0283 or PWD0587 (Table9) 1 hour prior to lethal toxin administration provided 80% survival. The TTM's of the non-surviving animals were 240 minutes and 124 minutes, respectively, for the anti-PA monoclonal antibodies. Administration of CAT002 by either route of administration as a negative control provided no protection against the lethal effects of systemic lethal toxin. All rats in the route-matched control groups exhibited the expected SYMPTOMS of animals exposed to toxic levels of PA/LF. TTM in the control animals ranged from 85 to 93 minutes.

Table 9

Group	N	Time of Study Agent relative to PA/LF (min)	Study Agent (3 mg/kg)	Route of Administration	PA (mg/kg)	LF (mg/kg)	% Survival	TTM (minutes)
1	5	-60	PWD0283	SC	0.09	0.032	0	145
2	5	-60	PWD0283	IM	0.09	0.032	80	240
3	5	-60	PWD0587	SC	0.09	0.032	0	105
4	5	-60	PWD0587	IM	0.09	0.032	80	124
5	5	-60	CAT002	SC	0.09	0.032	0	85
6	5	-60	CAT002	IM	0.09	0.032	0	93

Prophylactic Study 3: IV, SC and IM Administration of anti-PA Antibodies 24 Hours Prior to Injection of Lethal Toxin

[0387] Because administration of anti-PA antibodies given SC or IM 1 hour prior to lethal toxin was only partially protective, the experiment was repeated giving the anti-PA antibodies 24 hours prior to the lethal toxin, to allow the antibody more time to distribute in the animals. In this study, anti-PA antibodies were administered by SC, IM or IV injection 24 hours prior to a single intravenous injection of PA/LF. Male Fisher 344 rats (n = 5/treatment) were assigned to the groups shown in Table 10 Twenty-four hours before administration of PA/LF, animals received either PA mAb (PWD0283 or PWD0587), or a negative control mAb (CAT002).

[0388] As shown in Table 10, a single SC, IM or IV injection of PWD0283 or PWD0587 24 hours prior to injection of lethal toxin provided 100% survival at 24 hours with no apparent ill effects. In contrast, a single injection of the negative control mAb, CAT002, regardless of route of administration, provided no protection with 0% survival and an average TTM of 100 minutes. In a separate study, rats receiving anti-PA antibodies alone without PA/LF showed no adverse effects.

Table 10

Group	N	Time of Study Agent relative to PA/LF (min)	Study Agent (3 mg/kg)	Route of Administration	PA (mg/kg)	LF (mg/kg)	% Survival	TTM (minutes)
1	5	-24	PWD0283	SC	0.09	0.032	100	-
2	5	-24	PWD0283	IM	0.09	0.032	100	-
3	5	-24	PWD0283	IV	0.09	0.032	100	-
4	5	-24	PWD0587	SC	0.09	0.032	100	-
5	5	-24	PWD0587	IM	0.09	0.032	100	-
6	5	-24	PWD0587	IV	0.09	0.032	100	-
7	5	-24	CAT002	SC	0.09	0.032	0	88
8	5	-24	CAT002	IM	0.09	0.032	0	90
9	5	-24	CAT002		0.09	0.032	0	92

Prophylactic Study 4: Duration of Protective Effect of a Single IV administration of Anti-PA Antibodies against Multiple Lethal Toxin Challenge

[0389] The previous studies established that prophylactic administration of PWD0587 and PWD0283 protected Fisher 344 rats exposed to a lethal dose of anthrax toxin. The

following study was designed to establish if administration of anti-PA antibodies would provide protection with recurrent multi-day administrations of lethal toxin.

[0390] Male Fisher 344 Rats (F344) were randomly assigned to groups of 5 as shown in Table 11. One injection of PWD0283 or PWD0587 was injected intravenously 1 hour prior to the first PA/LF injection on Day 1. The dose of antibody administered was approximately 10-fold higher than the PA in a single dose of lethal toxin on a molar basis. To provide sufficient control animals for the duration of the experiment 65 rats received CAT002 on Day 1. To assure lethality of the toxin, 5 rats from the CAT002 group were dosed each day parallel to the dosing of the PA mAb groups. Lethal toxin was administered on subsequent days to the animals surviving from the previous day. This study reports the data for 11 injections of lethal toxin through 15 days.

[0391] On Day 1, a single injection of PWD0283 or PWD0587 60 minutes prior to injection of lethal toxin provided 100% survival, replicating the results observed in the previous studies. Moreover, the single injection of PWD0283 or PWD0587 continued to provide 100% protection after 11 subsequent lethal toxin injections over 15 days (Table 11). Administration of the negative control mAb, CAT002, provided no protection 0% survival) in any of the day-matched control groups. The TTM in the day-matched controls ranged from 79 to 113 minutes.

Table 11

Group	N	Time of Study Agent relative to PA/LF (min)	Study Agent (3 mg/kg)	Route of Administration	PA (mg/kg)	LF (mg/kg)	Day/ No. of PA/LF challenges	% Survival	TTM (minutes)
1	5	-24	PWD0283	IV	0.09	0.032	15/11	100	-
2	5	-24	PWD0587	IV	0.09	0.032	15/11	100	-
3	65	-24	CAT002	IV	0.09	0.032	15/1*	0	79-113

* Each set of 5 rats in Group 3, only received 1 challenge

[0392] Importantly, this study does not establish the maximum duration of the protective effect. Furthermore, repeated administration of PA/LF may have allowed the animals to develop their own protective immune response against PA. Indeed, daily administration of PA only, but not of LF only, for 14 consecutive days, allows rats to

survive a PA/LF challenge at day 14 (data not shown). This result is likely explained by the rats' generation of endogenous protective titers of neutralizing anti-PA antibodies.

Prophylactic Study 5: Protective duration of single IV administration of anti-PA monoclonal antibodies

[0393] The following study was designed to establish the duration of time which a single IV administration of anti-PA antibody would be protective against a single lethal toxin challenge.

[0394] Male Fisher 344 Rats (F344) were randomly assigned to groups of 5. Three, seven or fourteen days prior to PA/LF challenge, rats were given a single IV administration of PWD0283. Alternatively, seven, fourteen or twenty-one days prior to PA/LF challenge, rats were given a single IV administration of PWD0587. The dose of antibody administered was 1.5mg/kg, which is approximately 10-fold higher than the PA in a single dose of lethal toxin on a molar basis. Control rats were given the an isotype-matched, non-PA-binding, control antibody (CAT002). PWD0283 fully protected (100% survival) rats from PA/LF when given three days prior to lethal toxin challenge, and protected 60% of animals (as measured 24 hours following PA/LF challenge) when administered seven days prior to challenge. Administration of PWD0283 14 days prior to PA/LF challenge was not protective. PWD0587 fully protected (100% survival) rats from PA/LF (as measured 24 hours following PA/LF challenge) when administered seven, fourteen or twenty-one days prior to challenge. Control antibody was not protective at any time point.

[0395] Together, the results of prophylactic studies 4 and 5 demonstrate that in addition to being protective against single or multiple challenges of PA/LF, the antibodies of the present invention are useful, for example, as passive immunotherapy, until such time as an individual can develop endogenous protective anti-PA antibody titers through vaccination or infection.

Example 10: Dose Response of Prophylactic Monoclonal Antibody Treatment

[0396] Using the experimental approach defined in Example 8, Prophylactic Study 1 (with the exception that 0.09 mg/kg, rather than 0.16 mg/kg, of PA was injected in the PA/LF injection), the quantity of anti-PA antibody administered was titrated to determine the minimum amount of antibody that would be protective. It was determined that doses

of antibody equal to 10X, 1X, 0.75X, and 0.5X the molarity of PA injected allowed for 100% survival of animals, as measured 24 hours post PA/LF challenge. That is, doses as little as .075mg/kg (0.136 nanomoles) of either PWD0283 or PWD0587 were fully protective when the PA/LF challenge comprised 0.09 mg/kg of PA (.0272 nanomoles) and 0.032mg/kg of LF (0.089 nanomoles). Doses of PWD0283 and PWD0587 antibody at one-quarter the molarity (0.038 mg/kg) of PA injected allowed 80% and 60% of the animals challenged to survive at least 24 hours (n=5/group). Doses of antibody one-tenth the molarity (0.015 mg/kg) of PA injected were not protective, although TTM was slightly extended to approximately 150 minutes compared to animals injected with the control antibody, CAT002, where TTM was approximately 100 minutes.

Example 11: Efficacy of Anti-PA Monoclonal Antibody Against Aerosolized Anthrax in The Rabbit Model

[0397] The following study was designed to test the efficacy of an anti-PA antibody, administered as either a prophylactic or a therapeutic, of preventing or delaying death due to inhalational exposure to *Bacillus anthracis.*

[0398] New Zealand White Rabbits (2.5-3.5 kg from Covance, Inc.) were randomly assigned to six groups, each containing 12 animals evenly divided between male and female. Animals in each group were each challenged aerosol inhalation of anthrax spores, either after or prior to receiving anti-PA antibody or vehicle control. Group I was a control group that received no antibody treatment. Groups II-IV received a single prophylactic dose of 1, 5, 10, or 20 mg/kg of anti-PA antibody (PWD0587), respectively, administered subcutaneously 2 days prior to *B. anthracis* spore challenge. Group IV received a single therapeutic dose of 40 mg/kg of anti-PA antibody (PWD0587) administered intravenously one hour after *B. anthracis* spore challenge. Animals were exposed via aerosolization to a target dose of 100X LD_{50} of *B. anthracis* spores. Post-exposure measurements indicated that animals were actually exposed to spore challenge dose of 196X LD_{50}.

[0399] Observations: Clinical observations were recorded twice daily from receipt of rabbits until death. Body weights were recorded twice pre-dose, at dosing and at necropsy (data not shown). Food consumption (*ad libitium*) was confirmed by visual inspection. All animals that died or were euthanized due to moribundity had gross necropsy performed and recorded.

[0400] Blood collection: Blood collections were taken on days –7, 7, and 14 for hematology and serum chemistry as well as for determination of serum PA protein and anti-PA Antibody levels (results not shown). Serum PA protein and anti-PA antibody levels were also determined for blood collections taken at days 1, 2 and 4 (data not shown). Bacteremia was determined at Days 2, 7, 14 and at death (see below).

[0401] Study Termination: 14 days post-challenge, following blood collection, surviving animals were euthanized and a complete necropsy was performed and recorded. Tissue samples from all deaths (scheduled or unscheduled) were collected from liver, lung, mediastinal lymph node, spleen, kidney and brain.

[0402] Results: The efficacy of the treatments was assessed in three ways. First, the number of animals surviving at least 14 days post-challenge was recorded. Second, if animals died within the two weeks post challenge, the time to death was recorded. Third, the bacteremia of the blood at 2, 7 and 14 days post-challenge and at death was also assessed.

[0403] Results of this experiment are shown in Figure 6 and Table 1 below. All statistical tests are 2-sided and performed at the 5% level of significance.

[0404] The percentage of animals surviving to 14 days post-challenge among the vehicle control and PA mAb treated groups were compared using the Fisher's exact test. The survival at 14 days post-challenge is significantly different among the vehicle control and PA mAb treated groups (p-value < 0.0001, Table 1). Compared with the vehicle control group (survival at Day 14 = 0%), the survival at Day 14 is significantly higher in the 5.0 mg/kg sc group (42%, p-value = 0.0373), in both 10 mg/kg sc and 20 mg/kg sc groups (83%, p-value < 0.0001), and in the 40 mg/kg iv group (100%, p-value < 0.0001).

Table 12: Summary of the survival at Day 14 among all rabbits (N=12 per group)

Treatment	Survivors	P-value vs. Control [a]
Vehicle	0 (0%)	
1 mg/kg sc	0 (0%)	NA
5 mg/kg sc	5 (42%)	0.0373
10 mg/kg sc	10 (83%)	< 0.0001
20 mg/kg sc	10 (83%)	< 0.0001
40 mg/kg iv	12 (100%)	< 0.0001

[a] obtained from a 2-sided Fisher's exact test. The p-values for the comparison among all groups are < 0.0001, regardless of the inclusion or exclusion of the 40 mg/kg iv group in the analysis.

[0405] The Cochran-Armitage test was used to examine the dose response trend of the survival at 14 days post-challenge among the vehicle control and PA mAb sc treated groups. There is a significant dose-response trend with respect to the survival at day 14 (p-value < 0.0001). The percentage of animals of surviving to day 14 increase significantly as the dose level of PA mAb (PWD 0587) increases.

[0406] The survival time from spore challenge to death was analyzed using a log-rank test. The survival time for the rabbits that survived at the end of follow-up is censored at the 14-day study period. The survival time of the rabbits is significantly different among the vehicle control and PA mAb treated groups (p-value < 0.0001, Figure 1). Compared with the vehicle control group (median survival time = 2 days), the median survival times are 3 days in the 1mg/kg sc group (p-value = 0.0002), 6.5 days in the 5 mg/kg sc group (value < 0.0001), and more than 14 days in the 10 mg/kg sc, 20 mg/kg sc, and 40 mg/kg iv groups (all values < 0.0001), respectively.

[0407] The incidence of bacteremia in blood samples was also analyzed (See Table 13).

Table 13: Number of Animals with Bacteremia at Day 2, Day 7 or at Death

Treatment	Day 2	Day 7[A]	Death (see Figure 6)
Vehicle	12/12	NA	11/12 animals died on Day 2; 1 animal survived to Day 3
1 mg/kg sc	0/12	NA	12/12 animals died prior to Day 7; 10/12 animals were bacteremic at death
5 mg/kg sc	0/12	0/5	7/12 animals died on or prior to Day 7. 5 of those 7 animals were bacteremic at death
10 mg/kg sc	0/12	1/10	One animal died at day 6. A second animal died at day 7. Neither of these animals were bacteremic at death.
20 mg/kg sc	1/12[B]	1/10	One animal died at day 5. A second animal died at day 7. One of the animals was bacteremic at death.
40 mg/kg iv	0/12	0/12	12/12 animals survived to Day 14. No bacteremia was observed in 12/12 animals.

[A] Bacteremia of surviving animals indicated.

[B] Bacteremic animal at day 2 survived.

[0408] In summary, subcutaneous administration of anti-PA monoclonal antibody 2 days prior to lethal challenge with anthrax spores significantly prolongs time to death and/or increases the survival rate of challenged animals. Bacteremia was most often associated with found dead or moribund necropsied rabbits. Preliminary results showed no evidence of gross pathology at terminal necropsy.

Example 12 Cynomolgus Monkey Inhalation Spore Challenge Study

[0409] The following study was designed to examine the efficacy of an anti-PA monoclonal antibody, administered as a prophylactic treatment, against lethality due to inhalational exposure to *Bacillus anthracis* in cynomolgus monkeys.

[0410] 40 cynomolgus monkeys were randomly assigned to four groups, each containing 10 animals. Animals in each group were each challenged via aerosol inhalation of anthrax spores, 2 days after receiving anti-PA antibody (PWD0587) at 10, 20 or 40 mg/kg or vehicle control. Animals were exposed via aerosolization to a target dose of 100X LD_{50} of *B. anthracis* spores. Post-exposure measurements indicated that animals were actually exposed to spore challenge dose of 186X LD_{50}.

Statistical Methods

[0411] The primary efficacy endpoint is survival at Day 28 following spore challenge. Difference in 28-day survival between any one of the PA mAb treated groups and the vehicle control group are evaluated by 2-tailed Fisher's exact test. The secondary efficacy

endpoint is survival time, defined as the time from spore challenge to death during the 28-day study. The Cochran-Armitage test is used to examine the dose response trend among the groups.

[0412] The survival time from spore challenge to death was analyzed using a log-rank test. The survival time for the monkeys that survived at the end of follow-up is censored at the 28-day study period. All statistical tests are 2-sided and performed at the 5% level of significance.

Results

[0413] Figure 7 shows the percent survival of cynomolgus monkeys prophylactically treated with anti-PA monoclonal antibody PWD0587 (IgG1 format) and challenged with a lethal aerosolized dose of *B. anthracis* spores. Survival is significantly different among the vehicle control and PA mAb treated groups (P value = 0.0002). Compared with the vehicle control group, survival is significantly higher in the 10 mg/kg group (60%, P value = 0.0108), the 20 mg/kg group (70%, P value = 0.0031), and 40 mg/kg group (90%, P value = 0.0001) (Table 14). There is a significant dose-response trend with respect to survival (P value = 0.0002). The survival at Day 28 increases significantly as the dose level of PWD0587 increases.

Table 14 Survival in monkey study

Treatment	Survivors	P Value vs. Control [a]
Vehicle	0 (0%)	
10 mg/kg	6 (60%)	0.0108
20 mg/kg	7 (70%)	0.0031
40 mg/kg	9 (90%)	0.0001

[a] obtained from a 2-sided Fisher's exact test. The P value = 0.0002 for the comparison among all groups.

[0414] The survival time of the monkeys is significantly different among the vehicle control and PA mAb treated groups (P value < 0.0001, Figure 7). Compared with the vehicle control group (median survival time = 4 days), the median survival times in all 3 PWD0587 treatment groups are significantly longer (more than 28 days; all P values

≤0.0005), Figure 7. None of the surviving PA mAb-treated animals had positive (bacteremic) blood cultures at Days 7, 14, or 21 or 28.

Example 13: Detection of Neutralizing Antibodies Against Anthrax Protective Antigen by Edema Factor-Mediated cAMP Induction Assay

[0415] As described above, antibodies that neutralize the biological activity of PA protein can be identified by using a rubidium release assay such as the one described in Example 5 and/or a lethal toxin mediated cell killing assay such as the one described in Example 8. An additional assay which can be used to identify neutralizing antibodies against PA is an edema factor-mediated cAMP induction assay. This bioassay is based upon the ability of edema factor, a bacterial adenylate cyclase dependent upon PA for entry into cells, to bind PA and enter cells leading to a measurable increase in cAMP. In the presence of antibodies that neutralize PA, edema factor (EF) is inhibited from entering cells and reduced cAMP levels are observed.

[0416] Briefly, cells expressing anthrax receptor are exposed to edema toxin (PA+EF). Levels of cAMP in the cells are measured by any method known in the art, for example by ELISA using an anti-cAMP antibody. The ability of an antibody to inhibit edema toxin mediated increases in intracellular levels cAMP can be assayed by pre-incubating the edema toxin with a test antibody and then exposing the cells expressing anthrax receptor with the antibody/edema toxin mixture.

[0417] By way of non-limiting example, cAMP levels induced by edema factor, and the inhibition of same by anti-PA antibodies of the invention, can be measured using the following assay. 4000 Chinese Hamster Ovary cells in 100 microliters of CHO culture medium (CCM; F-12K growth medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 micrograms/ml streptomycin) are seeded into cAMP-Direct ELISA plates (Applied Biosystems, Foster City, CA; Cat No. T1507). Cells are incubated at 37°C, 5% CO_2, greater than 85% relative humidity while test samples are prepared.

[0418] In a separate assay plate, test antibodies starting at a concentration of 60 micrograms/milliliter in CCM/IBMX (CCM supplemented with 250 micromoles 3-isobutyl-1 methylxanthine) are diluted 3-fold for a total 10 serial dilutions in CCM/IBMX. 75 microliters of each dilution of antibody is then added to 75 microliters of PA/EF

solution (1200 nanograms/milliliter PA and 100 nanograms/milliliter EF in CCM/IBMX). anti-PA/edema toxin mixture is incubated at 37°C, 5% CO_2 for one hour.

[0419] At the end of the hour, the cell culture medium supernatant from the cAMP-Direct ELISA plates onto which CHO cells have been plated (described above), is removed. Then 100 microliters of the anti-PA/edema toxin mixture is added to the wells containing the cells which are then incubated at 37°C, 5% CO_2 for one hour. At the end of the hour, the anti-PA/edema toxin mixture is removed and 60 microliters of lysis buffer is added to the cells. From this point forward, the cAMP-Direct ELISA is completed according to the manufacturer's instructions. In the absence of neutralizing antibody, cAMP levels induced by edema toxin are approximately 100 fold greater than that induced by controls (e.g. samples containing no EF).

* * *

[0420] It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[0421] The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

[0422] Further, the Sequence Listing submitted herewith, in both computer and paper forms, is hereby incorporated by reference in its entirety.

[0423] The entire disclosure (including the specification, sequence listing, and drawings) of each of U.S. Provisional Application Nos. 60/391,162, filed June 26, 2002, 60/406,339, filed August 28, 2002, 60/417,305, filed October 10, 2002, 60/426,360, filed November 15, 2002, 60/434,807, filed December 20, 2002, 60/438,004, filed January 6, 2003, 60/443,858 filed January 31, 2003, 60/443,781, filed January 31, 2003, 60/454,613 filed March 17, 2003, and 60/468,651 filed May 8, 2003 is herein incorporated by reference in its entirety.